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Biophysical Chemistry 111 (2004) 197–204

Biophysical Chemistry

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The structure of DNA–DLPC–cationic gemini surfactant aggregates: a small angle synchrotron X-ray diffraction study

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Received 10 May 2004; accepted 26 May 2004
Available online 24 June 2004

Dedicated to Dipl. Ing. František Šeršeň PhD on the occasion of his birthday

Abstract

The structure of aggregates formed by interaction of DNA with unilamellar dilauroylphosphatidylcholine (DLPC) vesicles (DNA:DLPC = 1:1 base/mol) in the presence of gemini surfactant butane-1,4-diyl-bis(dodecyldimethylammonium bromide) (C12GS) was investigated using synchrotron small angle X-ray diffraction. In the concentration range $C12GS^+$:DLPC ≤ 1 mol/mol, a condensed lamellar L_α^c phase was found with a repeat period of lipid bilayer stacking in the range $d \sim 5.70-6.53$ nm and the DNA interhelical distance $d_{DNA} \sim 3.52-3.99$ nm, depending on the concentration of C12GS. At molar ratio $C12GS^+$:DLPC ≥ 0.35:1, the diffractograms have shown the presence of a second lamellar phase with the repeat period $d \sim 5.31$ nm which slightly decreases with increasing concentration of $C12GS^+$. The increasing fraction of this phase in the aggregates with increasing concentration of C12GS supports the association of this phase with microscopic domains enriched by surfactant molecules. The temperature behaviour of aggregates was investigated in the range 25–60 °C and the transversal thermal expansivities of the observed phases were determined.

Keywords: Gemini surfactants; Butane-1,4-diyl-bis(dodecyldimethylammonium bromide); Cationic vesicles; DLPC; DNA; Small angle X-ray diffraction

1. Introduction

The importance of the study of complex formation between DNA and cationic vesicles has now become evident due to growing interest in the use of cationic lipids [1–3], particularly quaternary ammonium surfactants [4], for the construction of liposomal genetic delivery systems. The basic requirements for effective transfection vectors are the ability to compact DNA, to protect them against degradation and to deliver them to the cell membrane with efficiency and specificity, and finally to facilitate the DNA transport through the cell membrane [5]. Three types of condensed organized cationic surfactant-lipid-DNA microstructures were identified: (i) spaghetti-like structures in which DNA is covered by a cylindrical lipid bilayer [6], (ii) honeycomb-like condensed columnar inverted hexagonal phase with linear DNA molecules surrounded by lipid monolayers forming inverted cylindrical micelles arranged on a hexagonal lattice [7] and (iii) condensed lamellar phase with ordered DNA monolayers intercalated between lipid bilayers [8]. The relationship between the structure of the complex and the transfection activity is intensively investigated for different novel synthetized cationic surfactants and lipid molecules [9,10].

The bis(quaternary ammonium) surfactants in which two cationic surfactant moieties are connected by a polymethylene chain, referred to as a spacer, are attracting increasing interest [11,12]. These surfactants show a very potent bactericidal activity [13,14], much larger than the conventional monoquaternary ammonium surfactants. Several papers deal with the correlation between structure, activity and critical micelle concentration of these surfactants [15,16], their interactions with lipid membranes [17–22],

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doi:10.1016/j.bpc.2004.05.012

and their ability to inhibit photosynthetic activity [23], to eliminate plasmids from bacterial host cells [24]. Their complexes with DNA were tested for transfection efficiency [25–28]. Ryhanen et al. [29] have observed different transfection efficiencies of binary vesicles composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine and cationic gemini surfactant, (2*S*,3*R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane dibromide (SR-1) as transfection vectors of pEGFP-N1 plasmid using multilamellar, unilamellar or only hydrated lipids dispersions for the preparation of cationic lipid–DNA aggregates. The highest efficiency was found for the aggregates prepared with multilamellar vesicles with high content of SR-1 gemini cationic surfactant, with a molar fraction of $X_{SR\text{-}1}>0.5$. These experiments support the importance of studying the structure DNA–cationic lipid aggregates formed also at high concentrations of surfactant molecules, out of the isoelectric point of complex.

In our previous work, we have found that multilamellar vesicles of dilauroylphosphatidylcholine (DLPC) with DNA in the presence of butane-1,4-diyl-bis(dodecyldimethylammonium bromide) (C12GS) at molar ratio DLPC:C12GS:DNA = 2:1:1.6 (mol/mol/base) form a condensed lamellar phase with ordered DNA monolayers intercalated between lipid bilayers ("sandwich" structure) [30]. The aim of the present work is study the structural changes of DNA + DLPC + C12GS aggregates formed by interaction of DNA with unilamellar DLPC vesicles at increasing concentration of C12GS, and the thermal behaviour of aggregates.

2. Materials and methods

2.1. Chemicals and sample preparations

Highly polymerized calf thymus DNA (Sigma, USA) at concentration 2 mg/ml was dissolved in 0.5 mmol/l HEPES buffer, pH ~ 7. The purity of DNA was checked spectrophotometrically; we have obtained $A_{260}/A_{280} = 1.8$. Butane-1,4-dyil-bis(dodecyldimethylammonium bromide), C12GS, was prepared by reaction of tertiary *N*,*N*,*N*′,*N*′-tetramethylbutane-1,4-diamine with 1-bromododecane as described in Ref. [13] and purified by manifold crystallization from a mixture of acetone and methanol. Aqueous solution of 0.01 mol/l C12GS was prepared in HEPES.

Dilauroylphosphatidylcholine (Avanti Polar Lipids, USA) at concentration 16 mg/ml was dispersed in HEPES buffer. The DLPC dispersion was vortexed and homogenized by sonication in an ultrasound bath and minimally threefold freezing–thawing process. From the dispersion of multilamellar vesicles, extruded unilamellar vesicles were prepared according to MacDonald et al. [31] using the LiposoFast Basic extruder (Avestin, Canada). The multilamellar vesicles were extruded through polycarbonate filter (Nuclepore, USA) with pores of diameter 50 nm mounted in the extruder fitted with two gas-tight Hamilton syringes (Hamilton, USA). The sample was subjected to 51 passes through the filter at room temperature.

DLPC unilamellar vesicles and C12GS solution were mixed at appropriate volume ratios to obtain the required molar ratio of C12GS:DLPC. The amount of lipid (2 mg) was kept constant in each sample. The sample was vortexed for a short time and DNA solution was added reaching the required ratio DNA:DLPC = 1:1 base/mol. The sample was again vortexed for a short time and the sediment created in the sample few minutes after preparation was placed between two Kapton foil (Dupont, France) windows of a sample holder for X-ray diffraction.

2.2. X-ray diffraction

Small (SAXD) and wide angle (WAXD) synchrotron radiation diffraction measurements were performed at the soft-condensed matter beam line A2 at HASYLAB at the Deutsches Elektronen Synchrotron (DESY) in Hamburg (Germany), using the monochromatic radiation of $\lambda = 0.15$ nm wavelength. The evacuated double-focusing camera was equipped with two linear delay line readout detectors. The SAXD detector was calibrated using silver behenate [32] and the WAXD detector by tripalmitate [33,34]. The sample was equilibrated at 20 °C (or other selected temperature) for 5 min before exposure to radiation. Temperature scans were performed at a scan rate 1 °C/min and the diffractograms were recorded for 10 s every minute. Data reduction and normalization were done with the programs STAFO and OTOKO (Refs. [35,36] and references therein). The diffraction peaks of SAXD region were fitted with Lorentzians and positions of maxima s_n ($n = 1, 2\ldots$), intensities I_n, integral intensities INT_n and half widths of peaks at one half of their intensity Δs_n were determined using a nonlinear least squares program. The WAXD pattern (not shown in the figures) of all measured samples exhibited one wide diffuse scattering characteristic for liquid-like carbon chains of phospholipid and C12GS molecules in the temperature range studied.

3. Results and discussion

3.1. The structure of DNA+DLPC+C12GS$^+$ aggregates

When a solution of cationic surfactant C12GS is added to the mixture of unilamellar DLPC vesicles, surfactant molecules intercalate between zwitterionic DLPC molecules and the surface becomes positively charged [37–42]. Unilamellar vesicles in the presence of C12GS do not show any diffraction peak in the region of SAXD because of the absence of a structure with a long-range organization. When DNA was added to the solution of C12GS + DLPC cationic vesicles, the formation of aggregates with long-range orga-

nization was observed. Typical SAX diffractograms observed for DNA + DLPC + C12GS aggregates are shown in Fig. 1. At low concentration of C12GS ($C12GS^+$:DLPC = 0.1:1 mol/mol), we have observed three reflections (Fig. 1a) identified as reflections of one-dimensional lamellar phase *La* of lipid bilayer stacking. The driving force for the interaction of DNA polyanion with cationic vesicles is the electrostatic interaction between negatively charged DNA phosphate fragments and positively charged quaternary ammonium groups of $C12GS^+$ molecules intercalated between DLPC molecules of the bilayer, enforcing close apposition of neighbouring vesicle bilayers. During the aggregate formation, both lipid and DNA undergo a complete structural transformation. When DNA helices form ordered monolayers between lipid bilayers, the structure of aggregate was designed as a condensed lamellar phase L_α^c [43], called as sandwich structure. The diffractogram of L_α^c phase exhibits 2–3 diffraction peaks characterizing a lipid bilayer stacking and usually one broad reflection from DNA–DNA correlations [30,44–46]. The absence of reflection from DNA stacking in our diffractogram (Fig.1a) indicates the topological disorder of DNA strands resulting probably from low surface charge density at low concentration of $C12GS^+$. At higher concentrations of $C12GS^+$ in the lipid bilayer, $C12GS^+$:DLPC ≥ 0.25:1 mol/mol, we have observed the reflection from DNA parallel stacking as indicated by arrows in Fig. 1b–f. Diffraction peaks were fitted by Lorentzians with linear background using a nonlinear least-squares method; the correlation of experimental points and fitted curves of the first order reflections are shown in Fig. 1a and b. The repeat period $d_{La} = 1/(s_{2a}\text{-}s_{1a})$ and the interhelical DNA distance $d_{DNA} = 1/s_{DNA}$ were determined from positions of maxima (s_{1a}, s_{2a}, s_{DNA}). At ratios $C12GS^+$:DLPC ≥ 0.35:1 mol/mol the first order reflections *L1a* have shown an increasing right shoulder asymmetry (Fig. 1c–d) that indicates the formation of second ordered structure in the aggregate. The observed splitting of the first and the second order reflections in the diffractogram at $C12GS^+$:DLPC = 0.75:1 (mol/mol) (Fig. 1e) clearly indicates the presence of the next lamellar phase *Lb* in the aggregates. At the highest studied molar ratio $C12GS^+$:DLPC = 1:1 (Fig. 1f), the *Lb* phase become dominant and the second order reflection of the *La* phase was practically unresolved from background. The SAX region at $s \sim 0.1–0.3$ nm^{-1} was fitted as a superposition of two or three Lorentzians (depending on the position of DNA reflection) as shows the inset in Fig. 1 with the $C12GS^+$:DLPC = 0.5:1 (mol/mol) diffractogram fitting. The dashed lines in Fig. 1c–f represent the Lorenzians of the first order maxima of the *La* and the *Lb* phase, respectively. The full lines in Fig. 1c–f are resulting from the sum of Lorentzians and linear background.

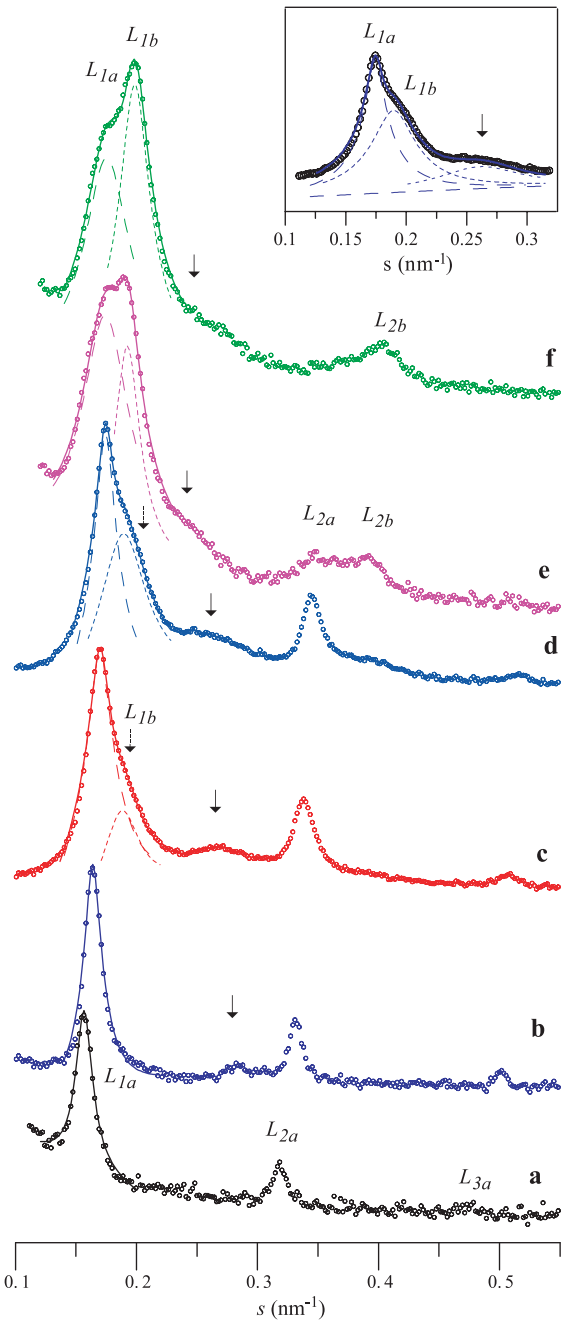

Fig. 1. SAXD patterns of DNA + DLPC + $C12GS^+$ aggregates at ratio DNA:DLPC = 1:1 base/mol and molar ratio of $C12GS^+$:DLPC: a) 0.1:1; b) 0.25:1; c) 0.35:1; d) 0.5:1 (detail in the inset); e) 0.75:1; f) 1:1 mol/mol at 25 °C. The arrows (full line) indicate the DNA reflection.

The repeat period of *La* phase and the interhelical DNA distance in dependence on the $C12GS^+$:DLPC molar ratio are shown in Fig. 2. With increasing concentration of $C12GS^+$ in the lipid bilayer, we have observed a nonlinear decrease of the repeat period of *La* phase, with the maximal change of d_{La}, $\Delta d_{La} = 0.73$ nm in the concentration range $0.1 \le C12GS^+$:DLPC ≤ 0.5 mol/mol; at higher molar ratios up to $C12GS^+$:DLPC ≤ 1 mol/mol, the repeat period only slightly decreased. The observed decrease of the repeat period is the result of electrostatic screening of the positive surface charge of the lipid bilayer due to interaction with negatively charged DNA phosphate fragments. Simulta-

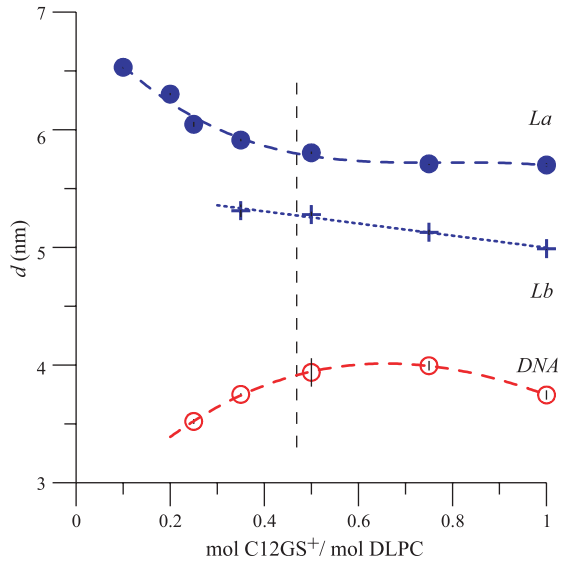

Fig. 2. Dependences of the *La* and *Lb* repeat periods and of the DNA interhelical distance on the molar ratio $C12GS^+$:DLPC. The dashed line indicates the isoelectric point.

neously with the decrease of repeat period, we have observed the changes in the lattice parameter d_{DNA} characterizing the DNA–DNA interhelical distance (Fig. 2). With increasing concentration of C12GS in the sample, the d_{DNA} increases nonlinearly from the value $d_{DNA} = 3.52 \pm 0.02$ nm at molar ratio $C12GS^+$:DLPC = 0.25 to the value $d_{DNA} = 3.99 \pm 0.04$ nm. The maximum change was observed in the interval $0.25 \leq C12GS^+$:DLPC ≤ 0.5 mol/mol. Depending on the lipid–cationic lipid mixture composition, the interhelical distances of DNA strands in the aggregates with sandwich structure were found in the range $d_{DNA} \sim 2.5$–6 nm [44]. Zantl et al. [47] have observed $d_{DNA} = 3.53$ nm at 45 °C in isoelectric DNA complexes with cationic DMPC + dimyristoyltrimethyl-ammoniumpropane (DMTAP) vesicles at DMPC:DMTAP = 1:1 molar ratio. The surface area of C12GS in egg yolk phosphatidylcholine bilayers estimated by X-ray diffraction is 0.69 ± 0.02 nm^2 with two cationic head groups [18,19]. This matches exactly the projected charge density of DNA—two anionic phosphate groups per 0.68 nm^2 [44]. The $C12GS^+$–DNA mixture has stoichiometric neutral charge when the number of $C12GS^+$ cations and DNA base pairs are equal; the isoelectric point of aggregates is thus $\chi_{iso} = n(C12GS^+)/n(DNAbp) = 1$, where $n(C12GS^+)$ and $n(DNAbp)$ is the number of $C12GS^+$ cation moles and DNA base pairs, respectively. The isoelectric point of aggregates recalculated on the molar ratio of $C12GS^+$:DLPC is shown in the Fig. 2 with a dashed line, supposing that all $C12GS^+$ molecules are intercalated in the DLPC bilayer. Fitting the dependence of $d_{DNA} = f$(mol $C12GS^+$/mol DLPC), we have obtained the interhelical DNA distance at isoelectric point of aggregate $d_{DNA} = 3.91$ nm. In our previous work [30] we have found $d_{DNA} = 3.64 \pm 0.02$ nm for DLPC:$C12GS^+$:DNA = 2:1:1.6 mol/mol/base aggregate formed with multilamellar DLPC dispersion. The DNA–DNA interhelical distance depends on $C12GS^+$:DNAbp charge ratio, but also on the volume fraction of DLPC. The molar ratio of $C12GS^+$:DLPC affects the lateral distribution of positive charge in the lipid bilayer surface. The gemini C12GS surfactant possess two cationic polar head groups which are connected by a spacer containing four carbon polymethylene chain. The distribution of positive surface charge and its lateral diffusion follows from the geometry and steric properties of molecules which are for gemini surfactants different in comparison with their monoalkyl analogues. In our experiments, we kept constant the molar ratio DNA:DLPC = 1:1 base/mol and in the range $C12GS^+$:DLPC ≥ 0.5 mol/mol the aggregates become positively charged. The observed increase of DNA interhelical distance with increasing of molar ratio $C12GS^+$:DLPC in Fig. 2 is probably the result of lateral electrostatic repulsion of head groups positive charge of adjacent $C12GS^+$ molecules intercalated in the DLPC bilayers. Lower packing density of DNA was observed also in the positively charged DNA + DOPC + dioleoyl trimethylammonium propane aggregates [44].

With increasing C12GS concentration the volume fraction of *Lb* phase increased (Fig. 1c–f). At molar ratio $C12GS^+$:DLPC ≥ 0.5:1 mol/mol, a broad shoulder of its first order reflection was partially superposed on the DNA reflection. The repeat period of the *Lb* phase was determined from the position of the first order diffraction maximum $d_{Lb} = 1/s_{1b}$. The dependence of repeat period d_{Lb} on increasing molar ratio $C12GS^+$:DLPC is shown in Fig. 2. We have observed a slight linear decrease of d_{Lb}. With the increasing content of C12GS in the sample the volume fraction of *Lb* phase increased, as demonstrated by the ratio of integral intensities INT_{L1b}/INT_{L1a} of the first order *La* and *Lb* reflections in Fig. 3. We have normalized the integral

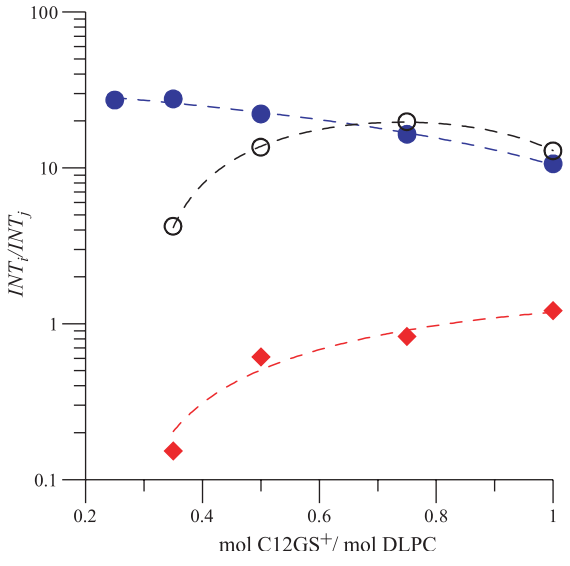

Fig. 3. Dependences of the first order reflection integral intensities ratio: INT_{L1b}/INT_{L1a} (♦); INT_{L1a}/INT_{DNA} (●) and INT_{L1b}/INT_{DNA} (○) on the $C12GS^+$:DLPC molar ratio.

intensity of the first order reflections of *La* and *Lb* phase by the integral intensity of DNA. The obtained dependences of integral intensities ratio INT_i/INT_j, where $i = L_{a1}$ or L_{b1} and j = DNA, document the slight decrease of volume fraction of *La* phase and the increase of *Lb* phase with increasing concentration of $C12GS^+$ intercalated in the DLPC bilayer (Fig. 3).

The observed repeat period d of lipid bilayer stacking is the sum $d = d_s + d_w$, where d_s is the steric thickness of a lipid bilayer and d_w the thickness of aqueous layer including DNA strands. We have observed the decrease of *La* repeat period in dependence on the $C12GS^+$ concentration in the range from $d_{La} = 6.53 \pm 0.01$ nm to $d_{La} = 5.70 \pm 0.01$ nm. The diameter of hydrated DNA is 2.4 nm [8]. Subtracting this value from the obtained repeat periods, we have obtained the ranges of steric DLPC + $C12GS^+$ bilayer thickness $d_{sLa} \sim 4.13-3.30$ nm. The steric thickness of lipid bilayer in unilamellar DLPC vesicles is $d_s = 4.16 \pm 0.19$ nm at 20 °C [48], and $d_s = 4.02 \pm 0.14$ nm in the DLPC lamellar phase [30]. Comparing these values with the d_{sLa} of the *La* phase, one can see a marked decrease of lipid bilayer thickness with increasing concentration of $C12GS^+$. The C12GS surfactant and lipid hydrocarbon chains have hydrocarbon chains of equal length, so the observed changes in the thickness are probably a result of lateral expansion of bilayer due to the increasing density of positive surface charge. Matti et al. [49] have observed a lateral expansion of the monolayers and an increase of ΔA/molecule of a cationic gemini surfactant, 2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane dibromide (SS-1) in mixtures with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) at molar fraction $X_{SS-1} > 0.5$ using a Langmuir balance in the presence of DNA at positively charged complex. At higher content of DNA (DNA:SS-1 = 3.5 charge ratio) this effect has not been observed.

The C12GS molecules itself at concentration range used in our experiments (below 0.2 wt.%) form spherical micelles [18]. The SAX diffraction pattern of DNA + C13GS mixture ($\chi = 0.6$) displayed one broad reflection with the maximum at $s = 0.26$ nm^{-1} and ill-defined shoulder at $s = 0.35$ nm^{-1}, what indicates an ordered structure with periodicity $d = 3.85$ nm in one dimension [50]. An aggregate formed due to interaction of DNA with C12GS micelles ($\chi = 13$) has shown at 20 °C also one broad reflection with maximum at $s = 0.24$ nm^{-1} indicating a structure with periodicity ~ 4.17 nm (our unpublished results). We have found the changes of the *Lb* phase repeat period from 5.31 ± 0.05 to 4.99 ± 0.01 nm, from which follows the steric thickness $d_{sLb} \sim 2.91-2.59$ nm. The obtained value of *Lb* phase steric thickness is too small for the phase formed only from DLPC molecules. The thickness of $C12GS^+$ polar head group region is smaller in comparison with the lipid polar head group region. We have found the thickness of DLPC bilayer hydrocarbon region $d_c = 2.22 \pm 0.04$ nm at 20 °C [30], what is approximately equal to the length of C12GS alkyl chain, and is close to the range of observed d_{sLb} values. To summarize the above mentioned data one can say, that the *Lb* phase has most probably its origin in the formation of microscopic domains more enriched by the $C12GS^+$ surfactant molecules in comparison to the *La* phase.

Typical SAXS scan of one-phase "sandwich" complex of DNA-cationic vesicles exhibit a set of sharp, periodically spaced reflections at $s_h = h/d$ and one broad peak at $s_{DNA} = 1/d_{DNA}$. The former are due to the lamellar bilayer–DNA structure with the period d and the latter to the ordered structure of DNA arrays with distance d_{DNA}. The DNA peak is broader than the lamellar ones. We have observed the changes of the DNA interhelical distance in the range $d_{DNA} \sim 3.52-3.99$ nm. Subtracting the diameter of hydrated DNA from these values, one calculates the distance between DNA molecules ~ 1.12–1.59 nm. This relatively large distance indicates the possibility of topological disorder in the DNA helical arrays. This disorder and thermally induced fluctuations contribute to the DNA peak broadening [36]. Fitting the SAX region of $C12GS^+$:DLPC = 1:1 mol/mol diffractogram at $s \sim 0.1-0.3$ nm^{-1} we have found $d_{DNA} = 3.75 \pm 0.04$ nm. There are two possible interpretations of this value: the decrease of the DNA arrays' lattice parameter follows from the DNA reorganization due to the higher volume fraction of *Lb* phase in the aggregate; or the obtained value is distorted by fitting procedure because of the intensive *L1b* reflection superposed on the broad DNA peak of low intensity.

3.2. Thermal behaviour of DNA+DLPC+$C12GS^+$ aggregates

Fully hydrated lipid bilayers are characterized by a large scale of fluctuations resulting in changes in intensities, widths and numbers of observable diffraction reflections [51]. An increase of temperature should induce an increased population of gauche rotamers in lipid acyl chains accompanied by a lateral expansion of the bilayer, what is manifested by lipid thickness decrease [52,53]. The increase of temperature also increases fluctuations of lipid bilayer which are accompanied by diffusion of water molecules from outside of lamellar phase in between lipid bilayers resulting in the increase of water layer thickness trapped between lipid lamellae [54]. Both phenomena mentioned above acting in the opposite directions are responsible for the observed repeat period of lamellar phase. The temperature dependence of the repeat period can be characterized by the transversal thermal expansivity α at the constant pressure π

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

where T is the absolute temperature and d is the repeat period. The DLPC multilamellar vesicles prepared in the same buffer as DNA + DLPC + $C12GS^+$ aggregates have

shown the increase of repeat period in the range 25–60 °C: $\alpha=(2.03\pm0.05)\times10^{-3}$ K^{-1} (Table 1).

We scanned the diffractograms of DNA + DLPC + $C12GS^+$ aggregates in the range 25–60 °C with aiming to study the thermal behaviour of both *La* and *Lb* phases. The intensity of the *La* phase first order reflection increases in the range 25–60 °C at molar ratios $C12GS^+$:DLPC ≤ 0.5:1; at higher molar ratios the intensity was constant within the experimental error (not shown). The volume fraction of the *Lb* phase formed at low concentration of surfactant decreased with increasing temperature. At molar ratio $C12GS^+$:DLPC = 0.35:1 mol/mol and temperature 60 °C the intensity of *L1b* peak was on the level of background (not shown). At molar ratio $C12GS^+$:DLPC = 0.5:1 mol/mol, this phase was present in the whole temperature range studied, however the ratio of integral intensities of the first-order reflections INT_{L1b}/INT_{L1a} decreased with increasing temperature (Fig. 4). The repeat periods of *La*, *Lb* phases and the DNA interhelical distance d_{DNA} of $C12GS^+$:DLPC = 0.5:1 mol/mol in dependence on temperature are shown in Fig. 4. With increasing temperature the repeat periods of the *La* and *Lb* phase decreased linearly and d_{DNA} has shown slight temperature fluctuations. The values of α obtained by least-squares fitting of $\ln d = f(T)$ for aggregates $C12GS^+$:DLPC = 0.25:1; 0.5:1 and 1:1 mol/mol are shown in Table 1.

As follows from Table 1, the transversal thermal expansivity of *La* phase decreased with increasing concentration of $C12GS^+$ in the lipid bilayer. The observed decrease of the repeat period is probably caused by the changes of the $C12GS^+$ + DLPC bilayer thickness. The DNA strands bind electrostatically to the $C12GS^+$ + DLPC bilayers and damp the thermally induced long-range bilayer fluctuations [30,55], increasing order in the structure and the diffusion of water molecules into the interbilayer space becomes restricted. The obtained transversal thermal expansivity of the *La* phase corresponds well with the thermal expansivity $\alpha=-(1.55\pm0.01)\times10^{-3}$ K^{-1} of DLPC:$C12GS^+$:DNA = 2:1:1.6 mol/mol/base aggregate formed from multilamellar DLPC dispersion [30]. Zantl et al. [47] have found transversal thermal expansivity (compression) $\alpha=-7.1\times10^{-4}$ K^{-1} of DMPC bilayer in L_α phase of DMPC + DMTAP + DNA at isoelectric point of the complex.

Table 1
The transversal thermal expansivities of the *La* and *Lb* phases and of the DNA interhelical distance in the DNA + DLPC + $C12GS^+$ aggregates at the molar ratio DNA:DLPC = 1:1 base/mol

$C12GS^+$:DLPC (mol:mol)	*t* (°C)	10^3 α (K^{-1})		
		La	*Lb*	DNA
0.25:1	25–53	−1.54 ± 0.03	–	0.89 ± 0.25
0.50:1	25–60	−1.80 ± 0.02	−2.23 ± 0.05	−0.47 ± 0.14
1:1	25–60	−2.48 ± 0.08	−1.08 ± 0.04	−2.78 ± 0.30[a]
0:1	25–60	2.03 ± 0.05		

[a] Determined in the range 25–50 °C.

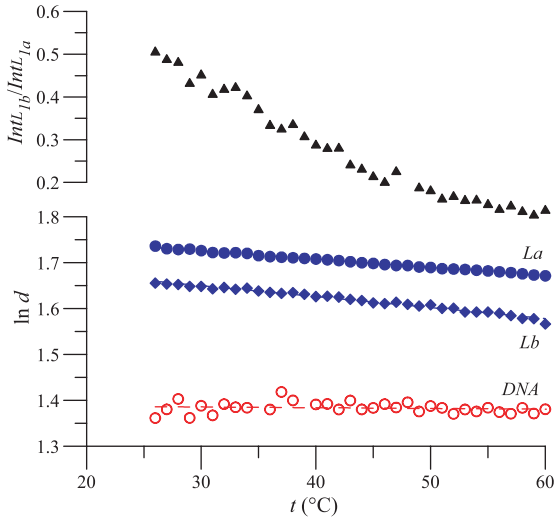

Fig. 4. Temperature dependence of the *La* and *Lb* repeat periods; the DNA interhelical distance and the first order reflection integral intensities ratio INT_{L1b}/INT_{L1a} of the DNA:DLPC:$C12GS^+$ = 1:1:0.5 base/mol/mol aggregate.

The repeat period of the *Lb* phase of $C12GS^+$:DLPC = 0.5:1 and 1:1 mol/mol aggregates also decreased linearly with increasing temperature in the range 25–60 °C. However, the transversal thermal expansivity increased with increasing concentration of $C12GS^+$ cations in the lipid bilayer. We suppose that the *Lb* phase is formed at higher content of $C12GS^+$ surfactant molecules in the lipid bilayer. In the process of heating, the temperature induced lateral expansion, undulations and changes in the bilayer bending modulus [51,54] can "dissolve" such domains to a certain content of surfactant molecules. We have observed such process of lateral reorganization of aggregate in $C12GS^+$:DLPC = 0.35 mol/mol sample. At higher content of C12GS in the sample the *Lb* phase was present in the whole temperature range studied. The small thickness of bilayer and the high density of $C12GS^+$ cations make most probably the interbilayer space easier accessible for water molecules in the process of heating.

The interhelical DNA distance has shown different temperature behaviour in dependence on $C12GS^+$:DLPC molar ratio. At low concentration of $C12GS^+$ in the lipid bilayer ($C12GS^+$:DLPC = 0.25:1 mol/mol), the interhelical distance increased in the process of heating [$\alpha=(0.89\pm0.25)\times10^{-3}$ K^{-1}] and probably this change follows the temperature induced lateral expansion of lipid bilayer. With the increasing concentration of surfactant we have found the decrease of interhelical DNA distance in the process of heating, indicating the formation of a structure with increased order. In our previous work [30] we have found the transversal thermal expansivity of d_{DNA} $\alpha=(0.36\pm0.11)\times10^{-3}$ K^{-1} of the DLPC:$C12GS^+$:DNA = 2:1:1.6 mol/mol/base aggregate formed with multilamellar DLPC dispersion. Zantl et al. [47] published $\alpha=1.6\times10^{-3}$ K^{-1} for DNA ordered in the DMPC + DMTAP + DNA isoelectric complex

in the L_α^c phase. This scatter of experimental values indicates that the thermal behaviour of DNA arrays follows the changes in the lateral distribution of positive charge of surfactant molecules intercalated in the lipid bilayer.

In the present experiments, we have observed the formation of the second lamellar phase in the aggregates at molar ratio $C12GS^+$:DLPC = 0.35:1 and $\chi = 0.75$. The aggregates were formed by adding DNA solution to the DLPC + C12GS unilamellar mixture stepwise. In our previous work [30] the DNA + DLPC + $C12GS^+$ aggregates formed in the mixture of multilamellar DLPC vesicles and the solution of C12GS was stepwise added to the DNA + DLPC mixture. At molar ratio $C12GS^+$:DLPC = 0.5:1 and $\chi = 1.23$ we have observed the sandwich structure of aggregate formed with one lamellar phase of the lipid bilayer stacking. As follows from these data, the structure of DNA-cationic lipids aggregates depends not only on stoichiometry of DNA-cationic lipid reaction but also on the structural organization of lipid molecules added (unilamellar or multilamellar vesicles) and on the method of preparation of aggregates. The isothermal titration calorimetry experiments [56] of plasmid DNA with a variety of cationic lipids also indicate on the possible formation of structurally different aggregates in dependence on the method of their preparation. It is important to know the structure DNA–cationic vesicles aggregates because one can expect that the geometry of the aggregate will influence the interactions between aggregates and cells, and thus the transfection process.

Acknowledgements

We thank Dipl. Ing. F. Šeršeň, PhD for many years of collaboration and for continuing interest in our work. This work was supported by the IHP-Contract HPRI-CT-1999-00040/2001-00140 of the European Commission (HASYLAB project II-02-016) and by the VEGA grants 1/0123/03 to PB and 1/0508/03 to FD.

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