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ARTICLE

Influence of poly(L-lysine) on the structure of dipalmitoylphosphatidylglycerol/water dispersions studied by X-ray scattering

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Abstract The interaction between the negatively charged phospholipid DPPG and positively charged poly(L-lysine) (PLL) of different lengths was studied by X-ray scattering in the SAXS and WAXS region. As a reference pure DPPG (Na salt) was investigated over a wide temperature range (−30 to 70°C). The phase behavior of DPPG in aqueous and in buffer/salt dispersions showed a metastable subgel phase at low temperatures and a recrystallization upon heating before reaching the liquid–crystalline phase. The presence of additional salt stabilizes the bilayer structure and decreases the recrystallization temperature. Large changes in the SAXS region are not connected with changes in chain packing. In DPPG/PLL samples, the PLL is inserted between adjacent headgroup layers and liberates counterions which give rise to a freezing point depression. In the complex with DPPG PLL form an α-helical secondary structure at pH 7 and temperatures below the gel to liquid–crystalline phase transition. This prevents DPPG from recrystallization and strongly increases the stacking order. The lamellar repeat distance is decreased and fixed by the helix conformation of PLL in the gel phase. PLL with $n = 14$ is too short to form helices and is squeezed out reversibly from the interbilayer space upon cooling by freezing of trapped water. In dispersions with longer PLLs ($n > 400$) at −20°C a 1D crystallization of PLL α-helices in the aqueous layer between the headgroups takes place. A structural model is presented for the lateral periodic complex, which is similar to the known cationic lipid/DNA complex.

Keywords DPPG · Poly(L-lysine) · X-ray diffraction · Peptide insertion · Complex building

Introduction

It was previously found that poly(L-lysine) (PLL) is a good model system to study the electrostatic interactions of polypeptides with negatively charged lipid bilayers because it is a positively charged polypeptide that is able to adopt all three common secondary structures, i.e., random coil, α-helix and β-sheet. The binding of PLL to negatively charged membrane surfaces was studied from early on. It was reported that long PLL increases the main transition temperature of phosphatidic acid (PA) membranes and induces phase separation in mixed membranes containing PA (Galla and Sackmann 1975; Hartmann et al. 1977; Hartmann and Galla 1978). When bound to PA membranes, PLL preferentially adopts the β-sheet conformation (Takahashi et al. 1996).

The negatively charged phospholipid phosphatidylglycerol (PG, Na^+ or NH_4^+ salt) is also often used as a lipid component to study the interaction of positively charged peptides with model membranes. The binding of PLL to PG membranes was studied by several groups (Papahadjopoulos et al. 1975; Takahashi et al. 1992; Carrier et al 1985). It was found (Carrier and Pézolet 1984, 1986; Fukushima et al. 1989) that at room temperature long chain PLLs are bound in an α-helical form,

Dedicated to Prof. K. Arnold on the occasion of his 65th birthday.

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whereas shorter PLLs cannot adopt this secondary structure. In addition, X-ray data indicated a change in chain order when long chain PLLs were present.

In recent papers (Durvasula and Huang 1999; Tenchov et al. 2001; Degovics et al. 2000), the lyotropic phase behavior of dipalmitoylphosphatidylglycerol (DPPG), which is often used as a model compound for negatively charged lipids, was re-investigated using different methods. The studies showed the importance of a correct pH to prevent a decomposition of the lipid. It became also clear that a strict control of the time-scale of the experiment is necessary because in some cases depending on the method and concentration of the sample long times are needed to reach equilibrium structures. It could be shown that thermal pretreatment of the sample leads to an increased molecular lateral order in the gel phase. In addition, it became evident that the ionic strength has a large influence on the phase transition behavior and on the structure of the gel phases. It became also clear that the binding of PLL to PGs has an influence on the phase structure of PGs. For instance, an incubation of DPPG with PLL at low temperature leads to the formation of an L_c-phase, but with a different structure than that of the L_c-phase of pure DPPG (Takahashi et al. 1992).

The goal of our study was to elucidate the phase behavior of DPPG in freshly prepared water suspension, DPPG in an aqueous buffer/salt suspension and DPPG in mixtures with PLL of different degree of polymerization over a wider temperature range. We will show that the use of a position sensitive detector covering the SAXS as well as the WAXS region and the display of the scattering data using contour diagrams as a function of temperature simplifies the analysis of the scattering data and yields additional information on the changes in packing of the lipids and the ordering of the PLLs as a function of temperature. In particular, we will show that at low temperature long chain PLLs can form a 1D array of α-helices intercalated in between the headgroup layers. This ordering is lost upon heating, though the α-helical structure of the PLL is retained as long as the DPPG is in the gel phase. Heating into the liquid–crystalline Lα-phase, however, leads to a loss of secondary structure of PLLs, but they remain bound to the negatively charged membranes.

Experimental

Materials

1,2-Dipalmitoyl-glycero-3-phosphoglycerol sodium salt (DPPG) was obtained from Lipoid KG (Ludwigshafen), poly-L-lysine hydro bromide (PLL) and all other chemicals were purchased from Sigma-Aldrich (Steinheim).

Sample preparation

From 10 mg DPPG a 20 mM DPPG/PLL suspension containing NaCl and phosphate buffer, pH 7, was prepared by addition of an equimolar amount of PLL solution. The mixture was homogenized by sonication and then lyophilized. For the diffraction studies the powder was resuspended in water. The amount of added water was adjusted to obtain a sample with a concentration of 50% (w/w) water and a concentration of 100 mM NaCl and 20 mM phosphate buffer. For comparison, the same solution preparation procedure was used for a pure DPPG suspension. In addition, a suspension of DPPG in freshly distilled water (pH 7) was investigated to clarify the influence of ionic strength.

X-ray diffraction

Powder patterns were measured in transmission with a stationary linear position sensitive detector ($2\theta = 0$–$40°$) on a stage including a curved primary Ge(111) monochromator and high temperature attachment (STOE & CIE GmbH Darmstadt). The samples were sealed in glass capillaries. Cu Kα1 ($\lambda = 0.154051$ nm) radiation was used, and the scattering was corrected with respect to an empty capillary. The X-ray patterns were combined in a single contour diagram to continuously present the scattered intensity from the SAXS to the WAXS region ($2\theta = 0$–$40°$, $s = 0$–$4.7\ \mathrm{nm}^{-1}$) between −30 and 70°C. The heating rate was 1/15 K min^{-1} (5 min equilibration, 10 min exposition for each pattern) for the applied temperature protocol.

Model display

Cerius^2 version 4.6 (Accelrys Software Inc.) was used for modeling and measurements. The created surface is the Connolly solvent accessible surface area.

Results and discussion

Polymorphism and structure of DPPGNa

The bilayer polymorphism of aqueous DPPG dispersions without additives (Fig. 1) is similar to that of the well-documented DPPC. A detailed analysis, however, shows that some peculiarities exist due to the existence

of the charged headgroup. In addition to the sub-, pre- and main-transition temperatures (T_{sub}, T_{pre}, T_m), metastable and recrystallized phases were observed (Table 1). Small temperature steps in the temperature protocol of the heating and cooling cycles and the display of the whole scattering intensity in reciprocal space up to a resolution of $s = 4.7\ nm^{-1}$ enabled us to analyze the thermal history dependent changes of pure DPPG as well as the influence of the different additives on the structure formation.

The scattering of DPPG in pure water is characterized by broad reflections in the WAXS and SAXS region, a few higher orders of the layer repeat distance, and a pronounced temperature dependence of the spacings (Fig. 1).

On cooling, a change in the short spacings without large change in the long spacings is observed at 14°C (T_{sub}) indicating a transition affecting only the chain packing mode. At 5°C (T_{ss}) a further transition is indicated by changes in the long spacings. In this case, however, the short spacings are unchanged. In a temperature region down to −10°C a two-phase region is observed. A decrease of the lamellar repeat distance from 7.25 to 5.71 nm is observed, accompanied by additional transformations. In this temperature region, probably changes in the head group conformation, position of the counter ions and hydration occur.

The appearance of special morphological features like cochleate cylinders (Garidel et al. 2001; Kodama et al. 1993) or bicelles (Meyer et al. 2001) was reported during annealing of DMPG at 4°C. However, the sub-gel phase Lc_m appearing at 14°C is metastable with respect to a stable Lc phase with a long-range lateral molecular order, which would produce additional short spacings in the range of 1.0–2.0 nm^{-1} (Raghunathan and Katsaras 1996; Wilkinson and McIntosh 1986; Blaurock and McIntosh 1986). In the metastable phase, mainly a spreading out of the fingerprint reflections appears (Fig. 1).

The appearance of ice reflections due to the freezing of trapped water gives rise to a further decrease of the layer repeat distance and also influences the chain packing of the lipid molecules (Förster and Brezesinski 1989). Up to eight sharp orders are observed and

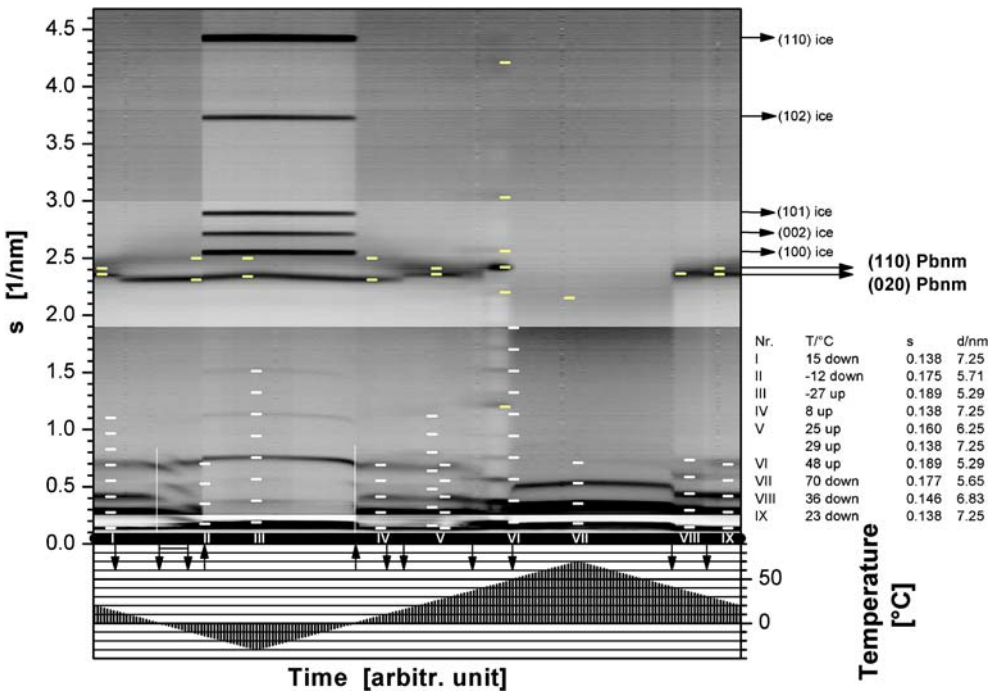

Fig. 1 X-ray contour diagram of an aqueous suspension of DPPG (Na^+ salt) prepared with pure water (pH 7). The scattering intensities are shown in the upper part as a function of reciprocal lattice spacing (*ordinate*) and temperature (*abscissa*). In the lower part the temperature course during the experiment is shown as a *ramp*. The *arrows pointing down* indicate the transition temperatures of the lipid in the up and down scans. The two *arrows pointing upward* indicate the onset of freezing and melting of water. In the temperature range between the arrows additional intense ice reflections are seen. Long spacings (different orders of the repeat distance) and respective short spacings (fingerprint scattering due to the aliphatic chain packing and sometimes additional spacings due to a molecular lattice) belong together at the selected temperatures I–IX and are indicated by horizontal short light *dashes*. Two light *vertical lines* are drawn at 0°C to illustrate the super cooling of water. Note the additional reflection at $s = 1.20\ nm^{-1}$ after recrystallization (VI) which is located as short spacing in between the long spacings (sixth and seventh order of the layer repeat distance)

Table 1 Layer repeat distances and phase transition temperatures of pure DPPG systems and DPPG/PLL mixtures evaluated from the analysis of X-ray powder patterns

Phases	DPPG + PLL			DPPG + buffer	DPPG + water
	$n = 1{,}181^{+}$	$n = 402$	$n = 14$		
Lc_m + ice (d_L/nm)	6.41	6.41	5.40	5.29	5.29
Lc_m (d_L/nm)	6.29	6.29	6.29	5.88	5.71
$L\beta_A$ (d_L/nm)	6.37	6.37	6.37	7.25	7.25/6.25
Cr (d_L/nm)	–	–	–	5.29	5.29
$L\alpha$ (d_L/nm)	5.88	5.88	5.88	5.13	5.65
T_{melt}/°C	–	–	–	50	50
$T_{recryst}$/°C	–	–	–	22	36
T_m/°C	50	49	45	43	40
T_{pre}/°C	–	–	–	33	31
T_{sub}/°C	19	20	18	16	15
T_{ss}/°C	–	–	–	7	1
ΔT_{ice}/K	−7	−8	−8	0	0

Lc_m metastable subgel phase, *$L\beta_A$* pseudo herringbone chain-packing mode in the gel phase, *Cr* recrystallized phase, *$L\alpha$* liquid–crystalline phase, *melt* melting, r*ecryst* recrystallization, *m* main-transition, *pre* pre-transition, *sub* sub-transition, *ss* solid–solid transition, ΔT_{ice} freezing point depression, + contour plot not shown

indicate a stack of well-ordered bilayers. The changed structure factors reflect the modification of the electron density profile.

The observed temperature dependence of the reflections on heating is caused by a successive pre-melting of ice and a small rehydration of the head groups (Kodama and Aoki 2001). At 0°C all ice melts and water is now entering the interbilayer space. The repeat distance reaches a value of 7.25 nm as initially observed. However, the initial chain-packing mode was different. Again a two-phase region is detectable at temperatures >8°C, in which the 7.25 nm repeat distance is retained. At T_{sub} the typical fingerprint scattering of the gel phase $L\beta_A$ appears. On further heating a recrystallization into a crystalline structure Cr takes place ($T_{recryst} = 37$°C), which transforms at $T_{melt} = 50$°C into the Lα phase. In the phase Cr the headgroups are probably dehydrated as concluded by the coincidence of the long spacings in the Cr phase with those observed at low temperature when the water has been frozen out (−30°C). However, the different structure factors indicate different electron densities within the same repeat distances. The recrystallized structure has an oblique subcell indicated by three fingerprint reflections, and the additional spacing at $s = 1.20\ nm^{-1}$ is taken as hint for a long-range order within a molecular lattice (Takahashi et al. 1992; Wilkinson and McIntosh 1986).

The observed appearance of metastable phases, a recrystallization phenomenon or the existence of oblique chain packing modes were recently reported for the diglyceride dipalmitoylglycerol (DPG) (Takahashi et al. 1999). Compared to the DPPG results reported by McIntosh et al. (Wilkinson and McIntosh 1986; Blaurock and McIntosh 1986) it seems that in their paper a subgel phase was obtained and analyzed, which is not identical with the recrystallized phase obtained here on heating. In the subgel phase of DPPC a molecular 2D order is observed (Raghunathan and Katsaras 1996), but not long-range 3D order like a crystalline phase. This is similar to DPPG. Because the melting temperature (Table 1) of the Cr phase is 8.5 K higher than the reported $T_m = 41.5$°C of the ripple phase of DPPGNa (Marsh 1990), it is evident that the phase Cr must be a stable crystalline phase.

On heating above 50°C the liquid–crystalline phase Lα is observed. The temperature dependence of the long spacing in the Lα phase is well understood: in the liquid–crystalline state the lateral expansion and vertical contraction is strongly influenced by thermal activation. On immediate cooling from the liquid–crystalline Lα-phase, the common polymorphism Lα–Pβ′–$L\beta_A$ appears.

From selected patterns of Fig. 1 the fingerprint reflections observed in the different phases were evaluated by a fit of the whole pattern. The results are shown in Fig. 2.

At 20°C the WAXS scattering is interpreted as consisting of a small sharp (020) peak and a large broadened (110) peak. This indicates a pseudo-herringbone chain-packing mode $L\beta_A$ within the symmetry *Pbnm* (Förster et al. 2000). This packing mode has a relative minimum on the energy hyper surface of the orthorhombic subcell and is a good model for the gel phase Lβ′ as suggested by Tardieu. The quite different line widths indicate tilted chains within uncorrelated bilayers (Tardieu et al. 1973). The recrystallized phase Cr has three fingerprint reflections of an oblique subcell (monoclinic or triclinic) and shows additional scattering

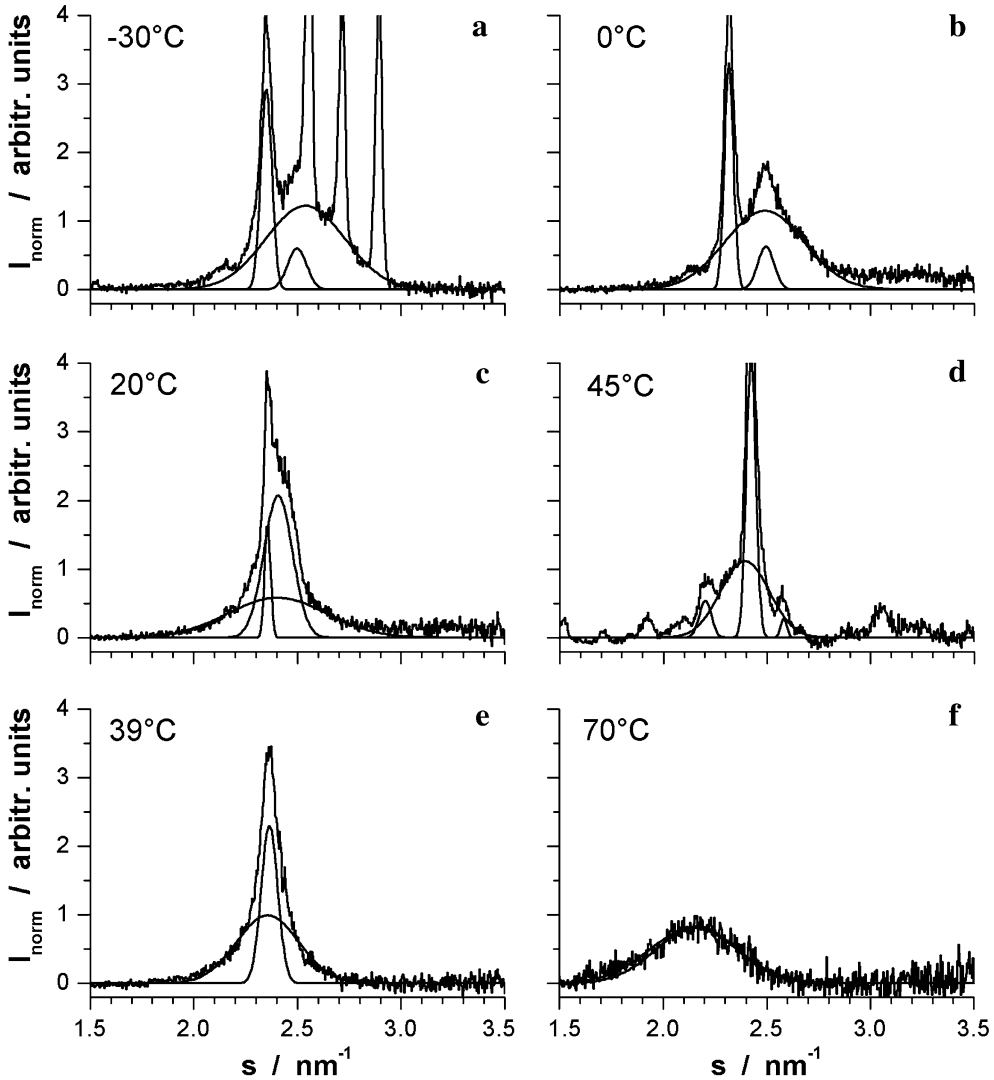

Fig. 2 Fitting results of the whole pattern: evaluation of the fingerprint reflections super positioned with a broad background halo in the phases. **a** Lc_m + ice, **b** Lc_m, **c** $L\beta_A$, **d** Cr, **e** $P\beta'$ and **f** $L\alpha$

(Fig. 1 VI) due to the occurrence of a hybrid subcell within a molecular lattice (Förster et al. 2001). In the metastable subgel phase Lc_m also three peaks were seen but fitted with quite different line width.

We now investigated the behavior of DPPG in the presence of buffer and additional NaCl (Fig. 3). The temperature behavior of the short spacing is the same but the SAXS reflections become sharper indicating a better bilayer stacking order. On cooling from room temperature again changes in the WAXS (13°C) and SAXS (8°C) are observed at the transition into the Lc_m phase. After supercooling and freezing of the trapped water, the orders of the layer repeat distance appear with their typical structure factor. On heating, the transitions appear to be fully reversible at the respective temperatures. The presence of additional salt does not lead to a melting point depression of the excess water. Only the recrystallization temperature for the phase Cr is decreased by about 15 K. The transition temperature into the $L\alpha$ phase is unchanged as well as the repeat distances which were measured for the different phases (Table 1). On cooling from the $L\alpha$ phase, the usual ripple phase $P\beta'$ appears again before the gel phase $L\beta_A$ is formed.

The large influence of temperature on the X-ray scattering intensities not only results in the melting of the chains and formation of liquid–crystalline phases, but also leads to changes in hydration and in the condensation of the ions at the interface of the bilayer. Hydration water can be released by the ions and the observed recrystallization is then induced. This was found before in DMPG alkaline earth cation systems (Garidel et al. 2000). It is therefore plausible that one effect of the salt containing buffer is to make the recrystallization easier.

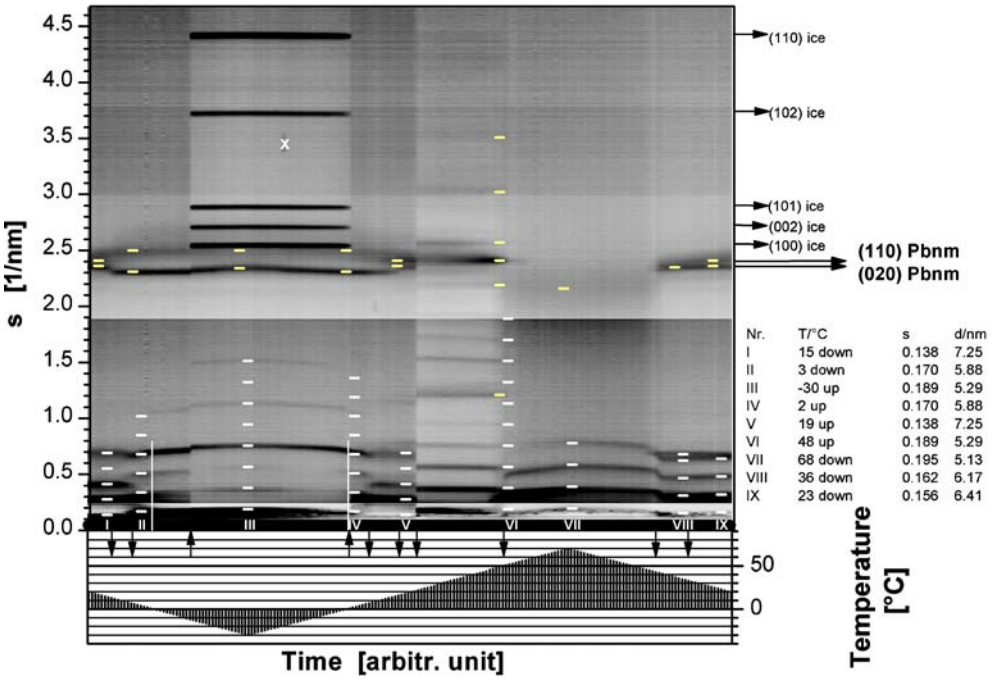

Fig. 3 Contour diagram of DPPG in an aqueous buffer/salt suspension. For explanations see Fig. 1

Influence of the poly(L-lysine) on the bilayer structure

PLL in different conformations were modeled using Cerius[2] and images of the shapes for $n = 14$ homologs are given in Fig. 4. The cross-section of a single β-strand is a rectangle 0.7×1.7 nm and that of a α-helix is a circle with a diameter of 1.8 nm. The helix diameter derived from the modeling is similar to the smallest measured PLL helix diameter within a hexagonal lattice (1.69 nm) measured in aqueous solution (Suwalsky and Llanos 1977). The lengths of the α-helices for different degrees of polymerization are 2.1 nm ($n = 14$), 60 nm ($n = 402$) and 176 nm ($n = 1181$), and the lengths of the respective β-strands are enlarged by the factor 4.27.

At pH 7, a random coil structure exists for PLL in aqueous solution (Greenfield et al. 1967). PLL in bulk solution at pH > 10.5 forms ordered secondary structures, when the side chains are deprotonated (Carrier

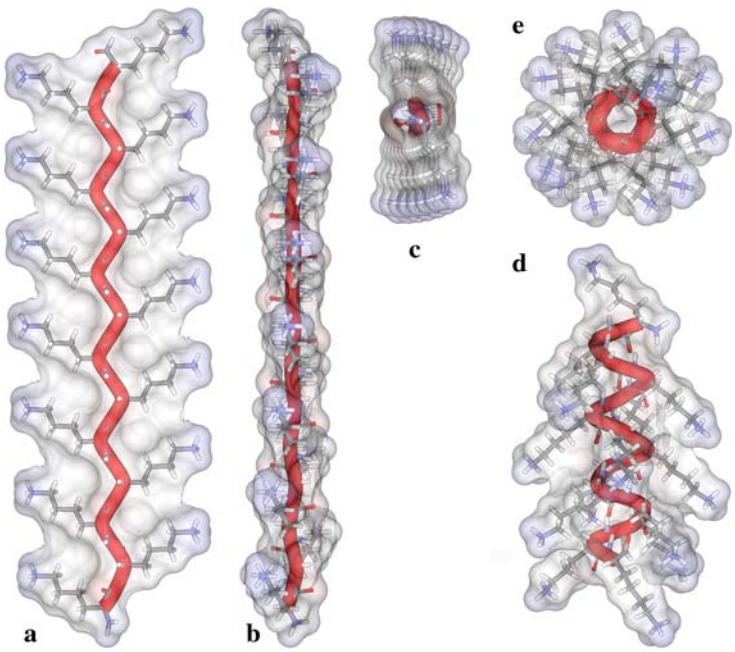

Fig. 4 Shape of a β-strand (**a** front view, **b** side view, **c** top view) and α-helix (**d** front view, **e** top view) of PLL ($n = 14$). The solvent accessible surface area is overlaid

et al. 1990). Dependent on temperature it adopts an α-helical (5°C) or an antiparallel β-sheet conformation as detected by FT-IR spectroscopy. At pH 7, the charged side chains prevent the formation of a defined secondary structure. When positively charged long chain PLLs are added to negatively charged DPPG gel phase bilayers, however, PLL adopts an α-helical conformation even at pH 7, as has be shown by FTIR-spectroscopy (Carrier and Pézolet 1986; Schwieger and Blume 2006). Only for $n = 14$ homolog, DPPG did not induce an α-helical conformation at temperatures below the main phase transition. With increasing temperature and decreasing PLL chain length, a random coil conformation is more favorable. In none of the mixtures, a β-sheet was detected by FTIR-spectroscopy (Schwieger and Blume 2006).

The addition of PLL to DPPG increases the stacking order of the bilayer strongly, even if the peptide has a low degree of polymerization. Up to nine higher orders of the repeat distance are detectable, and the repeat distance is only slightly temperature dependent, it remains almost constant even for the different gel phase polymorphs (Figs. 5, 6). The chain packing modes are not altered to a measurable extent by the addition of PLLs.

An increase of the repeat distance of about 0.5 nm and a change in the structure factor clearly indicate that PLL in the Lc_m phase is inserted in between adjacent head group layers. In all DPPG/PLL mixtures a freezing point depression of water was observed. It proves that the counterions are liberated by PLL binding and change the thermodynamic properties of excess water. As expected, this effect is independent of the chain length of poly(L-lysine). The sub-transition is not affected by the polypeptides, whereas, the main-transition temperatures increase with increasing molar mass of PLL (Table 1). For PLL ($n = 1{,}181$), it reaches a temperature (50°C) which is as high as the melting temperature of pure recrystallized DPPG. However, no recrystallization processes or pre-transitions occur in these DPPG/PLL mixtures.

In the Lα phase also an increase of the repeat distance by 0.75 nm is seen. However, for the gel phase, the layer repeat distance decreases by ca. 0.90 nm to values of 6.37 nm, which is reported as a characteristic value for DPPG in pure water without salt (Marsh 1990).

A change of the repeat distances in the measured range was also reported for the subgel phase of pure DPPG (Wilkinson and McIntosh 1986). It was concluded that for anionic lipids the repeat distance is fixed by the amount of ions and is limited by the added water. In the mixtures with PLL one can imagine that the inserted poly(L-lysine)bridges the adjacent headgroups after liberation of the counterions, which leads to a repeat distance similar to DPPG in pure water.

The phase behavior of the DPPG/PLL complexes depends on the length of the PLL. PLL with $n = 14$

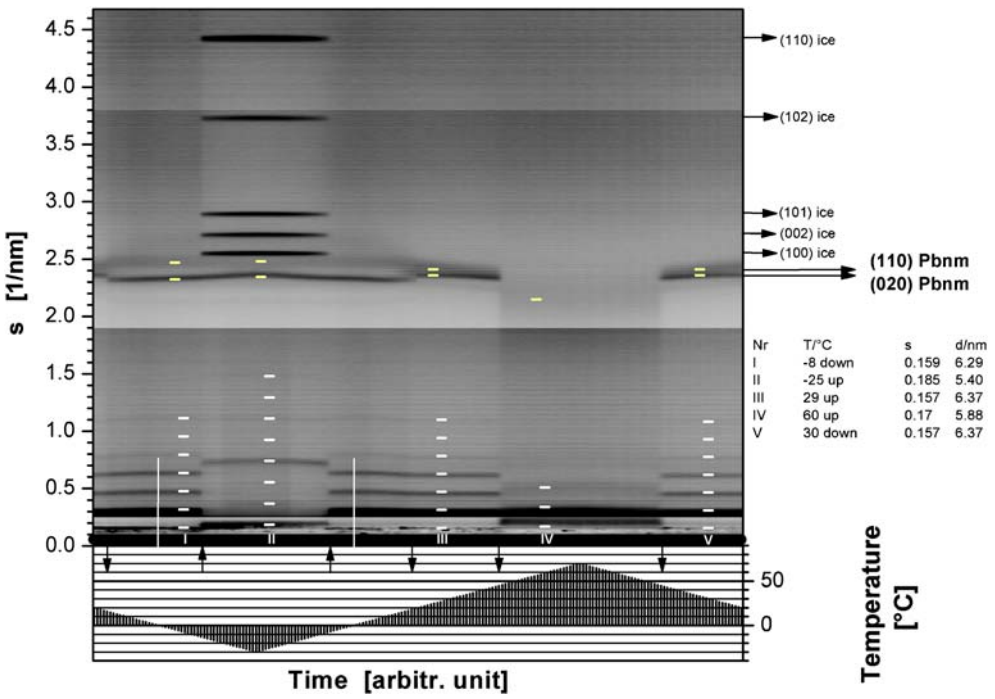

Fig. 5 Contour diagram of DPPG in a mixture with poly(L-lysine) with $n = 14$. For explanations see Fig. 1

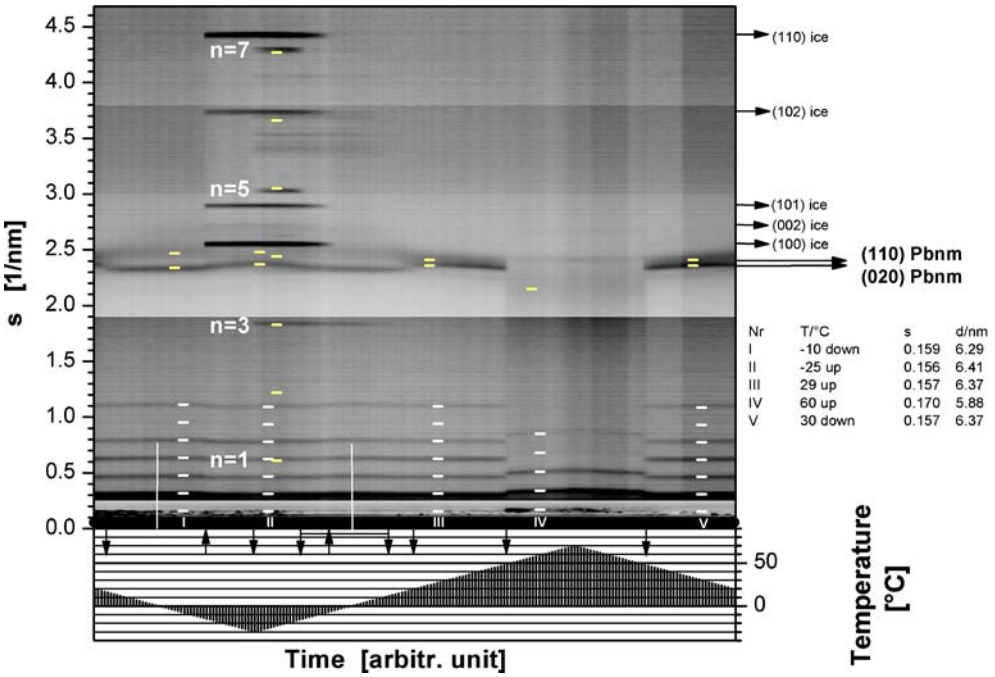

Fig. 6 Contour diagram of DPPG in a mixture with poly(L-lysine) with $n = 402$. Additional reflections appear at low temperatures. The strongest are marked by their orders of a 1.65 nm periodicity. The (002) ice reflection appears with low intensity. For further explanations see Fig. 1

shows the surprising effect that with the freezing of trapped water after supercooling, a repeat distance is observed which is characteristic for pure DPPG. Also the structure factor of the eight orders of the layer repeat distance is identical with that of pure DPPG. This indicates that short PLL is squeezed out from the interbilayer space during freezing of excess water and returns with the melting of ice. At high temperature in the liquid–crystalline Lα phase of DPPG, short PLLs remain bound to the headgroups in the interbilayer space but produce a stacking disorder which is concluded from the increased line broadening of the long spacings (Fig. 5).

In mixtures with longer PLL ($n > 400$) at low temperatures additional reflections appear in the WAXS region (Fig. 6). These are interpreted as caused by a 1D recrystallization of the PLL α-helices embedded between adjacent head group layers of opposing bilayers. The helices become stiff at low temperature and can then order in a parallel packing mode. Prerequisite for this process is an applied lyotropic stress, i.e., the freezing of free water. An increase of the repeat distance by 0.12 nm is observed before the helices pack in the 1D lattice at even lower temperature.

As mentioned above, only long chain PLLs form α-helices upon binding. Pure DPPG in aqueous suspension, in the presence of buffer/salt, and with short PLL molecules, shows d_L-values of 5.29, 5.29 and 5.40 nm, respectively, at low temperature when water has been frozen out. In each case reflections up to nine orders with a characteristic structure factor were observed. This is different for the mixture with PLL ($n = 402$). Here, the d_L-value is increased to 6.41 nm and the seven observable orders of the SAXS reflections have an altered intensity ratio indicating a different electron density profile of the lamellar structure. The freezing of the water does not have a large influence on the structure factor of the subgel phase. The appearance of additional WAXS reflections due to the ordering of the helices is also not connected with a change in the short spacings.

The evaluation of the additional reflections in the DPPG/PLL mixtures at low temperatures enabled us to create a model for a 1D periodic lateral packing of α-helices. The strongest reflections could be interpreted as the third, fifth and seventh order of a 1.65 nm periodicity (Fig. 6). Such a distance is characteristic for a 1D parallel packing of PLL helices, which was tested by modeling and was measured in the bulk (Suwalsky and Llanos 1977). The results of the modeling lead to the conclusion that a deformation and partial interdigitation of the side chains into the headgroup region are necessary to obtain the observed periodicity.

The results of our schematic modeling of the bilayer structure of the mixture are shown in Fig. 7.

The difference between the layer repeat distance and the helix periodicity yields a distance of 4.76 nm,

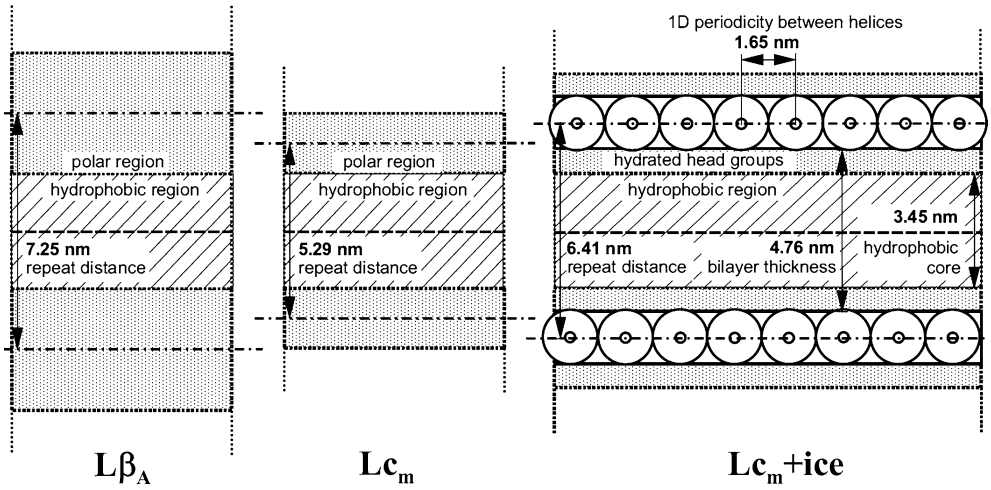

Fig. 7 Model for a DPPG/poly(L-lysine) complex illustrating the observed 1D helix periodicity in the metastable subgel phase Lc_m + ice. For comparison the bilayer structures of the phases $L\beta_A$ and Lc_m for pure DPPG (Na^+ salt) are also shown. The polar region consists of hydrated headgroups, counterions including buffer ions with their hydration shell, and water

which is correlated to the thickness of DPPG bilayer. It is consistent with values from the calculated electron density profile of a DPPG/PLL = 1/2 mixture (Takahashi et al. 1992). A distance of 4.46 nm between the phosphate groups across the bilayer was reported. The difference between both values (4.76 vs. 4.46 nm) is physically reasonable if one takes into account that the phosphate groups of DPPG carry glycerol moieties and that bound water is also still present. The thickness of the hydrophobic region can be set constant to 3.45 nm assuming a tilt angle of 32° for all DPPG phases (Blaurock and McIntosh 1986). This applies to all three lamellar structures with and without PLL as shown in Fig. 7.

At higher temperature, the α-helices are disordered when the gel phase in DPPG/PLL mixtures is reached upon heating. Similar results were obtained for other model polypeptides (Papadopoulos et al. 2004). For DPPG/PLL, the same layer repeat distance (6.37 nm) as observed for pure DPPG (Watts et al. 1981) is now found. This shows that electrostatics controls the equilibrium layer repeat distance of DPPG in both independently of the counterion nature.

The results show that poly(L-lysine) is inserted between adjacent headgroups, releases the counterions from lipid headgroups and has a stabilizing effect on the bilayer stacking in the gel phases Lc_m and $L\beta_A$. The released ions give rise to a freezing point depression of the excess water. Furthermore, the gel to liquid phase transition temperature is shifted to higher temperatures (Table 1).

Short PLLs (n = 14) are not able to form α-helices. The layer repeat distance in mixtures with short PLL in the phases Lc_m, $L\beta_A$ and Lα are the same as those observed for longer PLLs. Apparently the side chains are randomly oriented to both sides toward the headgroups of the DPPG layers and thus take up similar space (Fig. 4c, e). However, with the freezing of water short PLLs are excluded from the interbilayer space. For long chain PLLs the layer repeat distance is essentially constant in the gel phases and changes only when the Lα phase is reached. Here, the PLLs increased the repeat distance of the fluid lamellar phase by about 0.75 nm compared to pure DPPG in salt/buffer solution. This increase proves that PLL stay inserted in the interbilayer space even for fluid lipids. An analysis of the intensity of the reflections of all detectable orders yields differences due to the individual change of the electron density profiles by the inorganic ions or the short and longer PLL molecules. From FT-IR studies we know that the α-helices are only stable at the surface of gel state membranes and undergo a transition to random coil with the gel-to-liquid–crystalline phase transition (Schwieger and Blume 2006). The intensity differences of the reflections can therefore be interpreted as being caused by the different ordering of the PLLs in the water layer between the lipid layers.

Conclusions

In contact with negatively charged DPPG-membranes in the gel phase, long chain PLLs form an α-helix whereas shorter chain PLLs are bound as single strands in a random fashion. PLLs prevent DPPG

from recrystallization upon heating, the DPPG bilayers become flat and a dramatic increase in the stacking order is observed. While the long-range order is strongly changed, the PLLs do not change the chain packing modes within the bilayers.

When DPPG/PLL complexes are cooled below the freezing point of water two different effects were observed. Short chain PLLs were reversibly squeezed out from the interbilayer space when the freezing of water occurred. In the system with long chain PLLs, however, a 1D ordering of the α-helices with a specific distance was observed. Complexes with a similar geometry have already been found for the system of cationic lipids and anionic DNA (Rädler et al. 1997). Here, the double-stranded DNA is much stiffer so that the regular ordering of the double-helices is already present at room temperature. For the polypeptide system, the observation of ordered α-helices is new and can apparently only occur when the α-helices become stiff at low temperature concomitant with the freezing of water. At higher temperature, α-helices are still flexible enough so that this ordering is not possible (Papadopoulos et al. 2004). However, they remain bound to the lipid headgroups and are located in the interbilayer space.

References

Blaurock A, McIntosh TJ (1986) Structure of the crystalline bilayer in the subgel phase of dipalmitoylphosphatidylglycerol. Biochemistry 25:299–305

Carrier D, Pézolet M (1984) Raman spectroscopic study of the interaction of poly-L-lysine with dipalmitoylphosphatidylglycerol bilayers. Biophys J 46:497–506

Carrier D, Pézolet M (1986) Investigation of polylysine-dipalmitoylphosphatidylglycerol interactions in model membranes. Biochemistry 25:4167–4174

Carrier D, Dufourcq J, Faucon J F, Pézolet M (1985) A flourescence investigation of the effects of polylysine on dipalmitoylphosphatidylglycerol bilayers. Biochim Biophys Acta 820:131–139

Carrier D, Mantsch H, Wong P (1990) Protective effect of lipidic surfaces against pressure-induced conformational changes of poly(L-lysine). Biochemistry 29:254–258

Degovics G, Latal A, Lohner K (2000) X-ray Studies on aqueous dispersions of dipalmitoyl phosphatidylglycerol in the presence of salt. J Appl Crystallography 33:544–547

Durvasula RV, Huang CH (1999) Thermotropic phase behavior of mixed-chain phosphatidylglycerols: implications for acyl chain packing in fully hydrated bilayers. Biochim Biophys Acta 1417:111–121

Förster G, Brezesinski G (1989) Lyotropic stress in lipid–water model systems. Structural changes induced by freezing of the solvent. Liq Crystals 5:1659–1668

Förster G, Meister A, Blume A (2000) Van der Waals energy contour map describing the orthorhombic hydrocarbon chain packing of symmetry *Pbnm*. Phys Chem Chem Phys 2:4503–4508

Förster G, Meister A, Blume A (2001) Chain packing modes in crystalline surfactant and lipid bilayers. Curr Opin Coll Interf Sci 6:294–302

Fukushima F, Muraoka Y, Inoue T, Shimozawa R (1989) Conformational study of poly(L-lysine) interacting with acidic phospholipids vesicles. Biophys Chem 34:83–90

Galla HJ, Sackmann E (1975) Chemically induced lipid phase separation in model membranes containing charged lipids: a spin label study. Biochim Biophys Acta 401:509–529

Garidel P, Förster G, Richter W, Kunst BH, Rapp G, Blume A (2000) DMPG divalent cation complexes: an X-ray scattering and freeze-fracture-electron-microscopic study. Phys Chem Chem Phys 2:4537–4544

Garidel P, Richter W, Rapp G, Blume A (2001) Structural and morphological investigations of the formation of quasi-crystalline phases of 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG). Phys Chem Chem Phys 3:1504–1513

Greenfield N, Davidson B, Fasman GD (1967) The use of computed optical rotatory dispersion curves for the evaluation of protein conformation. Biochemistry 6:1630–1637

Hartmann W, Galla HJ (1978) Binding of polylysine to charged bilayer membranes. Molecular organization of a lipid–peptide complex. Biochim Biophys Acta 5:474–490

Hartmann W, Galla HJ, Sackmann E (1977) Direct evidence of charge-induced lipid domain structure in model membranes. FEBS Lett 78:169–172

Kodama M, Aoki H (2001) Water behavior in phospholipid bilayer systems. Surf Sci Series 93:247–293

Kodama M, Miyata T, Yokoyama T (1993) Crystalline cylindrical structures of Na^+-bound dimyristoylphosphatidylglycerol as revealed by microcalorimetry and electron microscopy. Biochim Biophys Acta 1168:243–248

Marsh D (1990) CRC handbook of lipid bilayers. CRC Press

Meyer HW, Richter W, Rettig W, Stumpf M (2001) Bilayer fragments and bilayered micelles (bicelles) of dimyristoylphosphatidylglycerol (DMPG) are induced by storage in distilled water at 4°C. Colloids Surf A 183–185:495–504

Papahadjopoulos D, Moscarello M, Eylar EH, Isac T (1975) Effects of proteins on thermotropic phase transitions of phospholipid membranes. Biochim Biophys Acta 401:317–335

Papadopoulos P, Floudas G, Klok HA, Schnell I, Pakula T (2004) Self-assembly and dynamics of poly-(p-benzyl-L-glutamate) peptides. Biomacromolecules 5:81–91

Rädler JO, Koltover I, Salditt T, Safinya CR (1997) Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. Science 275:810–814

Raghunathan VA, Katsaras J (1996) Lbeta′ Lc′ phase transition in phosphatidylcholine lipid bilayers: a disorder-order transition in two dimensions. Phys Rev E 54:4446–4449

Schwieger C, Blume A (2006) Interaction of poly(L-lysines) with negatively charged membranes—an FT-IR and DSC study. Eur Biophys J (in this issue)

Suwalsky M, Llanos A (1977) An X-ray diffraction study of poly(L-lysine hydrobromide). Biopolymers 16:403–413

Takahashi H, Matuoka S, Kato S, Ohki K, Hatta I (1992) Effects of poly(L-lysine) on the structural and thermotropic properties of dipalmitoylphosphatidylglycerol bilayers. Biochim Biophys Acta 1110:29–36

Takahashi H, Yasue T, Ohki K, Hatta I (1996) Structure and phase behaviour of dimyristoylphosphatidic acid/poly(L-lysine) systems. Mol Membr Biol 13:233–240

Takahashi H, Watanabe M, Quinn PJ, Hatta I (1999) Simultaneous X-ray diffraction and calorimetric study of metastable-to stable solid phase transformation of 1,2-dipalmitoyl-sn-glycerol. Biophys Chem 77:173–181

Tardieu A, Luzzati V, Reman FC (1973) Structure and polymorphism of the hydrocarbon chains of lipids. Lecithin-water phases. J Mol Biol 75:711–733

Tenchov B, Koynova R, Rapp G (2001) New ordered metastable phases between the gel and subgel phases in hydrated phospholipids. Biophys J 80:1873–1890

Watts A, Harlos K, Marsh D (1981) Charge-induced tilt in ordered-phase phosphatidylglycerol bilayers. Evidence from X-ray diffraction. Biochim Biophys Acta 645:91–96

Wilkinson DA, McIntosh TJ (1986) A subtransition in a phospholipid with a net charge, dipalmitoylphosphatidylglycerol. Biochemistry 25:295–298