Eur Biophys J (2007) 36:437–450
DOI 10.1007/s00249-006-0080-8

ARTICLE

Interaction of poly(L-lysines) with negatively charged membranes: an FT-IR and DSC study

Christian Schwieger · Alfred Blume

Received: 8 May 2006 / Accepted: 20 June 2006 / Published online: 16 August 2006

Abstract The influence of the binding of poly(L-lysine) (PLL) to negatively charged membranes containing phosphatidylglycerols (PG) was studied by DSC and FT-IR spectroscopy. We found a general increase in the main transition temperature as well as increase in hydrophobic order of the membrane upon PLL binding. Furthermore we observed stronger binding of hydration water to the lipid head groups after PLL binding. The secondary structure of the PLL after binding was studied by FT-IR spectroscopy. We found that PLL binds in an α-helical conformation to negatively charged DPPG membranes or membranes with DPPG-rich domains. Moreover we proved that PLL binding induces domain formation in the gel state of mixed DPPC/DPPG or DMPC/DPPG membranes as well as lipid remixing in the liquid–crystalline state. We studied these effects as a function of PLL chain length and found a significant dependence of the secondary structure, phase transition temperature and domain formation capacity on PLL chain length and also a correlation between the peptide secondary structure and the phase transition temperature of the membrane. We present a system in which the membrane phase transition triggers a highly cooperative secondary structure transition of the membrane-bound peptide from α-helix to random coil.

Keywords DPPG · Poly(L-lysine) · Peptide binding · Secondary structure · Domain formation

Abbreviations

DPPC	1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine
DPPG	1,2-Dipalmitoyl-*sn*-glycero-3-phosphoglycerol
DMPG	1,2-Myristoyl-*sn*-glycero-3-phosphoglycerol
PS	Phosphatidylserine
PA	Phosphatidic acid
PLL	Poly(L-lysine)
DSC	Differential scanning calorimetry
FT-IR	Fourier transform infrared spectroscopy
CD	Circular dichroism
NMR	Nuclear magnetic resonance
ESR	Electron spin resonance
FRET	Fluorescent resonance energy transfer
FCS	Fluorescence correlation spectroscopy

Introduction

Electrostatic interactions at lipid membrane surfaces play an important role in binding of protein to membranes and have been intensively studied using a variety of techniques. In many proteins, agglomerations of positively charged amino acids (mainly lysines and arginines) are found that provide an electrostatic binding domain for the negatively charged plasma membranes (Heimburg et al. 1999; Montal 1999; Wang et al. 2004). It was found that poly(L-lysine) (PLL) is a good model system for cytolytic and antimicrobial peptides to study the electrostatic interactions (Shai 1999; Blondelle et al. 1999) because it is a highly positively charged polypeptide that is able to adopt all three common secondary structures, i.e. random coil,

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

C. Schwieger · A. Blume (✉)
Institute of Physical Chemistry, Martin-Luther-University Halle-Wittenberg, Muehlpforte 1, 06108 Halle, Germany
e-mail: alfred.blume@chemie.uni-halle.de

α-helix and β-sheet. That has been proven for bulk solution (Greenfield et al. 1967; Jackson et al. 1989) as well as for membrane surfaces containing negatively charged lipids (Hammes and Schullery 1979; Fukushima et al. 1988; Carrier and Pézolet 1984). It was found vice versa that PLL influences many membrane properties. It was reported that long PLL increases the main transition temperature of PA membranes (Galla and Sackmann 1975; Hartmann and Galla 1978; Takahashi et al. 1996) as well as of PG membranes (Papahadjopoulos et al. 1975; Takahashi et al. 1992; Carrier et al. 1985). On the other hand it was found that the transition temperature of lipopolysaccharide (LPS)-containing membranes was not been affected by the addition of PLL (Lasch et al. 1998) and that short PLLs (ca. 20 monomer units) even decreased the transition temperature of membranes containing PA (Laroche et al. 1988) or PG (Carrier and Pézolet 1986). This shows that the interaction of PLL with negatively charged lipids is strongly dependent on the nature of the lipid headgroup and on the chain length of the PLL itself. Furthermore it was reported that PLL has influence on the lamellar-hexagonal phase transition (De Kruijff and Cullis 1980), membrane curvature (Dolowy 1979), fusion rates (Gad et al. 1985), vesicle adhesion (Menger et al. 2002), membrane rupture (Diederich et al. 1998) and permeability (Yaroslavova et al. 2003). It has even been described that PLL of a certain chain length might even be translocated through the membrane, probably using defects produced upon binding (Menger et al. 2003; Shibata et al. 2003). Of outstanding importance is also the notion of domain formation in mixed lipid membranes induced by the binding of PLL. Domain formation has been shown by ^{2}H-NMR (Franzin and Macdonald 2001), fluorescence (Carrier et al. 1985), FT-IR spectroscopy (Lasch et al. 1998), Raman spectroscopy (Carrier and Pézolet 1984), ESR (Galla and Sackmann 1975) and electron microscopy (Hartmann et al. 1977) for different membrane mixtures. However, in most of the studies, statements can only be made about gel phase domains because different transition temperatures are used as indicator for different domains. Although some methods, namely NMR, ESR or fluorescence techniques (FRET, FCS), are capable of detecting domains in the fluid membrane state, measurements have rarely been done above T_m. A lipid demixing in the fluid membrane state that would be of biological relevance has not been shown up to now.

Besides publications on the influence of PLLs many studies have been performed on the interaction of oligolysines with negatively charged membranes (Roux et al. 1988; Kim et al. 1991; Mosior and McLaughlin 1992; Loura et al 2006), including theoretical studies on the thermodynamic of binding (Ben-Tal et al. 1996, 2000; Denisov et al. 1998; Murray et al. 1999). These authors show that the binding of oligolysines is of pure electrostatic nature and occurs only peripherally with a membrane peptide equilibrium distance of approximately 2.5 Å, indicating that at least one layer of water separates it from the membrane. However, for PLL binding it is still unclear whether hydrophobic interactions are involved in the binding and whether the PLL might penetrate the headgroup region (Hartmann and Galla 1978; Carrier et al. 1985). The experimental results obtained for oligolysines differ from those of PLL, in that no domain formation induced by the oligolysines could be observed. This finding might be connected to the inability of oligolysines to form defined secondary structures.

The motivation of our work arises from the feeling that though many principal findings for PLL binding to negatively charged membranes were already published by several groups, a systematic study of the thermotropic behaviour of PGs and its dependence on PLL chain length is still missing. Especially extensive DSC studies were not presented up to now. We focused in our investigation on DPPG as a negatively charged membrane component and varied the PLL chain length in six steps from 14 to 906 monomer units as well as the lipid-to-peptide mixing ratio (R_i) and the membrane composition. Furthermore, we correlated the thermotropic behaviour of the DPPG/PLL complexes with the secondary structure of the membrane-bound PLL, which we recorded first in the whole temperature range from gel to liquid–crystalline phase. It turned out that not only the secondary structure influences the phase behaviour of the membrane, but that the phase state of the membrane also determines the secondary structure of the bound peptide.

We will show, based on FT-IR spectroscopic results, that long-chain PLLs are bound as α-helices to gel phase DPPG but gradually convert to a random coil structure when the sample is heated into the liquid–crystalline phase of the lipid. This transition from an α-helix to a random coil becomes highly cooperative when DPPG is mixed with the neutral DPPC. In this case the membrane phase transition triggers the cooperative secondary structure transition of the membrane-bound peptide. We will also show that in the mixed system phase separation of neutral and charged lipid components can be induced by long-chain PLLs.

Experimental

Materials

Poly(L-lysines)

The PLLs with degrees of polymerization ranging from 14 to 906 (determined by viscosity) were purchased from Sigma-Aldrich (Steinheim, Germany) and used without further purification. Bromide was used as counter ion. PLL concentrations are given in mol lysine monomer/l.

Lipids

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol were a gift from Nattermann Phospholipid GmbH (Cologne, Germany) and used without further purification. D-62-DPPC was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Lipid mixtures were prepared by mixing solutions of the lipids in $CHCl_3$/MeOH (2:1) and evaporating the solvent under a slight stream of nitrogen at a temperature of 50°C. Remaining traces of solvent were removed at 50°C under vacuum overnight.

Vesicle preparation

For DSC experiments, pure or mixed lipids were dispersed in aqueous solution containing 110 mM NaCl by cyclic heating over the phase transition temperature and repeated vortexing. Vesicles were then sized by extrusion through a 100 nm polycarbonate membrane using a LiposoFast-Extruder (Avestin). Vesicle size was determined by dynamic light scattering using an ALV-NIBS/HPPS spectrometer (ALV-Laser Vertriebsgesellschaft m.b.H., Langen, Germany). For IR spectroscopic measurements lipids were dispersed in 110 mM NaCl solution and sonicated at temperatures above phase transition temperatures. Vesicle preparations were stored in the refrigerator at ca. 4°C.

Methods

DSC

Differential scanning calorimetry was performed with a Microcal VP-DSC (MicroCal Inc., Northampton, USA). In all experiments we used a heating rate of 1°C/min and a time resolution of 4 s. Lipid and peptide samples were prepared separately and mixed directly before measurement. The lipid concentration in the calorimetric cell was always 0.25 mM. Peptide concentration varied according to the desired R_i. Reference was always 110 mM NaCl solution. At least three up- and down scans were performed for each sample to prove the reproducibility. All presented curves originate from the second heating scan.

FT-IR spectroscopy

Spectra were recorded using Bruker Vector 22 spectrometer (Bruker GmbH, Germany) equipped with a DTGS detector. PLL–lipid samples were placed between two CaF_2 windows, which were separated by a Teflon spacer 56 μm in thickness. The hollow sample mount was thermostated by an external circulating water bath (Haake F3 C, Gebr. Haake GmbH, Karlsruhe, Germany). Temperature was incremented in steps of 2°C and equilibrated for 8 min after each step. Thirty-two scans with a spectral resolution of 4 cm^{-1} were collected and Fourier transformed after one level of zero filling. Lipid and peptide samples were prepared separately and mixed directly on the CaF_2 window before measurement. The lipid preparations were used in a concentration of 67 mM. For experiments in D_2O solution both the lipid and the peptide preparations were lyophilized twice and resuspended in D_2O. Mixtures of perdeuterated and non-deuterated lipids were measured using a 25 μm spacer. For data processing, the Bruker OPUS FT-IR software was used. Spectra of a 110 mM NaCl solution in H_2O or D_2O, respectively, were used as reference and subtracted from the sample spectra. Peak positions were determined by the second derivative method.

Results and discussion

DSC studies

DPPG membranes

In the course of this study we performed several series of DSC experiments on the system of negatively charged membrane systems with PLL under variation of (1) the PLL chain length, (2) the lipid-to-peptide mixing ratio (R_i) and (3) the membrane charge density. The latter was adjusted using mixtures of zwitterionic DPPC or DMPC with zero net charge and negatively charged DPPG.

The DSC curves in the range of the $L_{\beta'} \rightarrow L_\alpha$ phase transition of DPPG membranes complexed with equimolar amounts (with respect to charges) of PLL are shown in Fig. 1. The endothermic transition seen in the

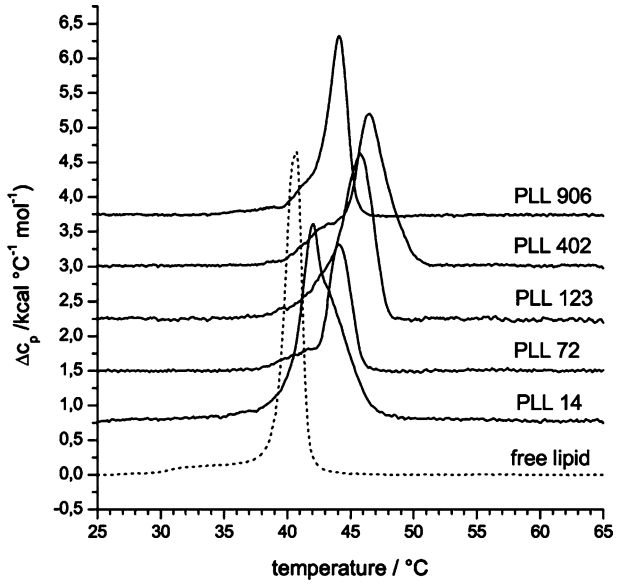

Fig. 1 DSC plots of the gel-to-liquid-crystalline phase transition of DPPG/PLL complexes with an equimolar charge ratio ($R_i = 1$) and different PLL chain lengths

DSC scans is the so-called main transition and the associated temperature is the main transition temperature, T_m. In DPPG multilamellar systems also a $P_{\beta'}$-phase exists and the so-called pre-transition is seen below the main transition (Schneider et al. 1999). However, in small unilamellar vesicles this transition is usually not resolved or even absent (Heimburg 2000). The dotted line represents the phase transition of the uncomplexed DPPG vesicles. The other curves show the phase transitions of DPPG/PLL complexes formed with PLL of different chain lengths. It is obvious that T_m of the complexes is increased with respect to the free DPPG membrane. The value of increase is dependent on the chain length of the PLL. While the shorter peptides, PLL 14 and PLL 72, cause a shift in T_m of a little more than 1°C, the longer peptides (PLL 123–906) produce a shift of 4–6°C. In general, T_m seems to increase with the chain length of the absorbed PLL. However, we find a lower T_m for the complex DPPG/PLL 906 than for the complex DPPG/PLL 402. This is in contrast to the general trend. The general rise in main transition temperature indicates a stabilization of the gel phase ($L_{\beta'}$) upon binding of PLL. As the negative charges of the membrane are screened by oppositely charged PLL and consequently the electrostatic repulsion between neighbouring DPPG molecules is reduced, this may result in better packing and higher van der Waals attractions between the lipid molecules, thus leading to a higher T_m. (The effect on the hydrocarbon chain packing is discussed by Förster et al. 2006.) The chain length dependence is obviously caused by different binding constants of the PLL chains to the lipid vesicles. In general, the binding constant increases with the chain length of the peptide, which is not an enthalpic but essentially an entropic effect (Montich et al. 1993). Although the mobility of the bound peptide is confined (Ben-Tal et al. 2000), the system gains a much higher entropy by counter ion release (Wagner et al. 2000). Monovalant ions are released during the complexation of two polyelectrolytes, as which both, membrane and PLL, can be understood. Before binding, counter ions (chloride and sodium) are bound to the polyelectrolyte and therefore restricted in their translational degrees of freedom. After binding, those ions are released and may move freely in solution. This leads to a gain in translational entropy, which is the main driving force of binding (May et al. 2000). The effect is larger when the number of associated and, thus during the binding, released counter ions is higher. This explains why the stabilization of the gel phase and consequently the rise in T_m is more pronounced for PLL 402 than for PLL 14. An additional gain in entropy comes from the release of water molecules from the binding sites (Lehrmann and Seelig 1994; Garidel and Blume 1999). The sharing of water of hydration between PLL and vesicle membrane is another effect which is responsible for the increase in T_m.

The lower transition temperature of the complex DPPG/PLL 906 compared to DPPG/PLL 402 can be explained by steric effects. For very long peptide chains it is more difficult to bind to the membrane surface in a way to get maximal coverage. This is consistent with a model of two-dimensional packing of stiff cylinders (Novellani et al. 2000), which claims that the porosity of a package rises with the cylinder length. Overall we see two competitive effects with increasing peptide chain length: the increase of the binding constant and the larger steric hindrance. These two competitive effects lead to a maximal T_m for a PLL with intermediate chain length, namely PLL 402.

The same experiments were repeated with lipid and with peptide excess concentrations ($R_i = 2$ and 0.5, respectively). The results are shown in Fig. 2. The general finding of an increase in T_m after PLL binding remains valid for these conditions. But in contrast to the case of $R_i = 1$, the half-width of the transition peaks gets considerably wider and the peaks get structured in different components, which are apparent as shoulders and side peaks. This indicates that several consecutive transitions occur in the system. Triphasic transitions were already observed by Papahadjopoulos et al. (1975), Carrier et al. (1985) and Carrier and Pézolet

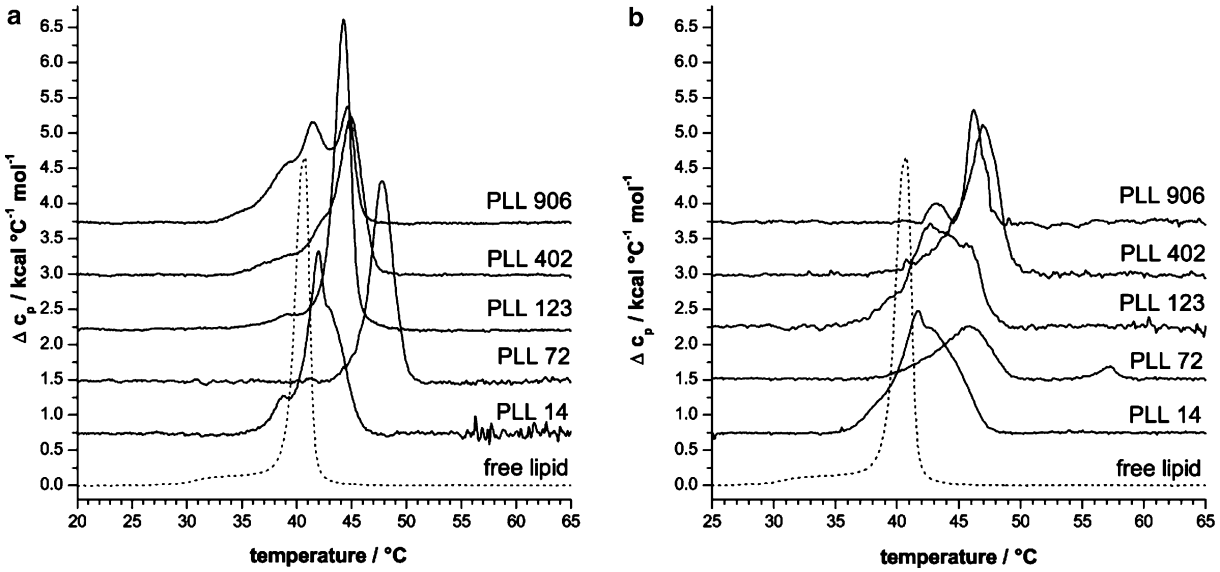

Fig. 2 DSC plots of the gel-to-liquid-crystalline phase transition of DPPG/PLL complexes with a mixing ratio of **a** $R_i = 0.5$ (PLL excess) and **b** $R_i = 2$ (DPPG excess)

(1986). These findings were interpreted as being caused by domain formation in the membrane or by heterogeneity of the complexes. The tendency to form domains is more pronounced in the complexes with longer peptides. Consequently PLL 906 induces the largest splitting of the transition peak. The chain length dependence in the domain-forming capacity is also reported by other authors (Franzin and Macdonald 2001; Macdonald et al. 1998). Furthermore it was found that the transition peaks do not shift in a continuous fashion. This means that domains form in more or less well-defined structures. That could be completely uncovered membrane regions (T_m about 41°C), completely covered membrane in a 1:1 stoichiometry or intermediate structures. Also the secondary structure of the peptide (which is discussed later) should have an influence on the structure of the domain. In case of an equimolar mixing ratio (Fig. 1) domain formation is less probable because every lipid molecule is screened by the same electrostatic field and there are no charge differences throughout the membrane.

Mixed DPPG/DPPC and DPPG/DMPC membranes

The pure negatively charged DPPG membrane is a simple model system, but biologically less relevant. We therefore performed additional experiments with mixed membranes by adding zwitterionic DPPC or DMPC to negatively charged DPPG. Thus the surface charge density of the membrane is reduced, which will have effects on binding constants, saturation concentrations and steric effects during binding. In addition, negatively charged and neutral lipids can demix and domain formation gets even more probable compared to pure DPPG membranes (Russ et al. 2003). Demixing of membrane lipids might play an important role in physiological processes and was already described for different lipid mixtures (Franzin and Macdonald 2001; Denisov et al. 1998; Heimburg et al. 1999; May et al. 2000).

Figure 3a shows DSC plots of mixed DPPG/DPPC membranes (1:1) complexed with PLLs of different chain lengths in a mixing ratio $R_i = 1$ (R_i refers to the DPPG component in the lipid mixture). Again, a general rise in main transition temperature after PLL binding is observed. The maximal transition temperatures (ca. 44.7°C) are lower than for the binary DPPG/PLL mixtures (ca. 47°C). This is expected because the DPPC component is uncharged and does not bind PLL (data not shown). Only the DPPG binds PLL and its transition is shifted. The chain length dependence is more pronounced than in the case of pure DPPG membranes. As we observe a continuous increase in T_m with increasing chain length, we conclude that an increasing binding constant dominates over unfavourable steric interactions. This effect is to be expected because in the mixed membrane we have less binding sites per area, which gives the PLL more space to pack at the surface. Furthermore, we observe an increasing half-width of the transition peaks with increasing polypeptide chain length. This is an indication that domain formation occurs, which is more pronounced in the complexes with longer PLL. The domain formation leads now to the separation of free DPPC, the remaining DPPG molecules with bound PLL having a higher transition

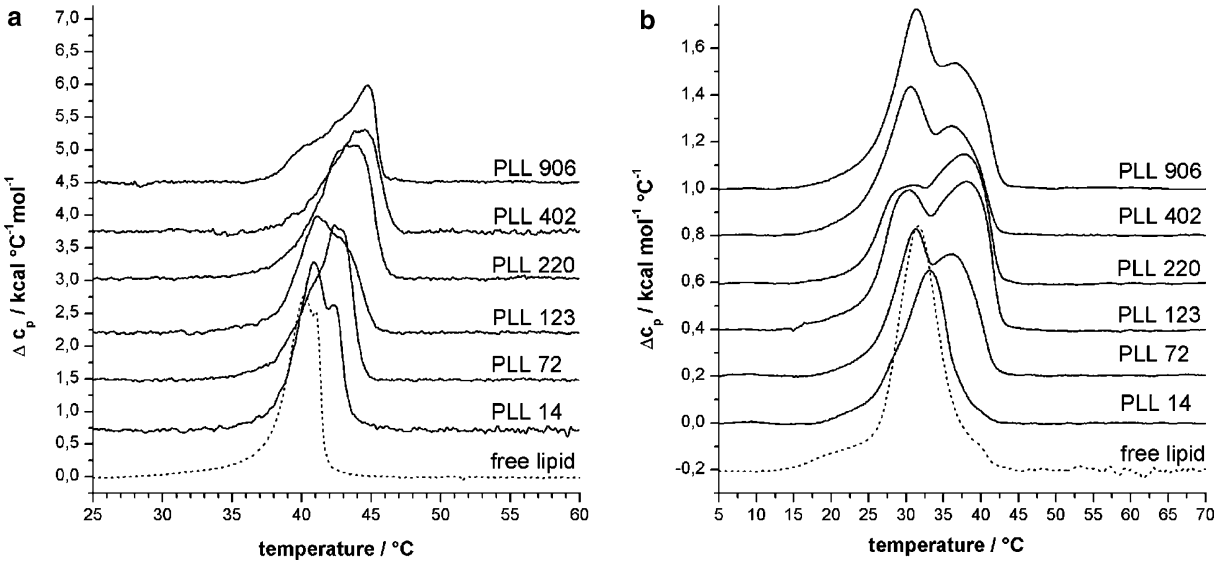

Fig. 3 DSC plots of the complexes of PLL of different chain lengths with **a** DPPG/DPPC (1/1, mol/mol) and **b** DPPG/DMPC (1/1, mol/mol). The charge ratio R_i = 1 and 0.5, respectively

temperature. However, the separated domains will not be pure DPPC or DPPG, respectively, but only enriched in one of the components. The domains seem to become larger with longer PLL chain length, as indicated by the change of the DSC peaks. Macdonald et al. (1998) suggested that the area of the domains increases proportionally to the square root of the polyelectrolyte molar mass. However, the domains also get less defined in their composition because the PLL packing gets less ideal and more porous, which leads to wider transition peaks. Similar results were described by Franzin and Macdonald (2001) for membranes containing PS. They measured a smaller PS accumulation in domains induced by longer PLL.

For DPPC/DPPG mixtures the domain formation is not easy to observe because the pure components have the same transition temperature. Therefore, we also investigated mixtures of DPPG with DMPC. These two components mix ideally (Garidel et al. 1997) and a 1:1 mixture has a transition into the liquid–crystalline phase that occurs at a temperature of 31°C, in between that of the pure components (DMPC 24°C, DPPG 41°C). This DMPC/DPPG mixture has therefore the advantage that the transition peaks of DPPG- and DMPC-enriched domains will be much better separated. The results of the experiment of addition of PLL to this lipid mixture are shown in Fig. 3b. After addition of PLL the peaks split into two components at about 30–31 and 35–38°C. The unequal distance from the original transition peak shows that the peptide binding domains are much more enriched in DPPG, than the free ones in DMPC.

To study the influence of complex composition on T_m and on the domain formation, we made a series of experiments with various amounts of PLL bound to mixed DPPC/DPPG membranes. The properties should change with R_i up to the isoelectric point and beyond that we would expect a stable saturated complex. However, charge overloading with charge reversal is also possible (May et al. 2000). According to Franzin and Macdonald (2001) domain formation should be most favourable for high lipid contents ($R_i > 1$) and long PLL chains. The results for complexes with the shortest and longest PLL are shown in Fig. 4. In Fig. 4a the transition peaks for complexes with PLL 14 are rather narrow. Either there is no domain formation or the domains are to small to undergo a cooperative phase transition at increased T_m and can thus not be observed by the DSC experiment. The influence of R_i on the domain formation is small. Under similar experimental conditions other authors (Franzin and Macdonald 2001; Laroche et al. 1988; Carrier et al. 1985) also could not detect domain formation for short PLLs either with PG- or with PA or PS-containing membranes. Nevertheless, small molecules, as pentalysine (net charge 5) or spermine (net charge 4), are also able to induce lipid segregation at lower ionic strength of the solvent (Denisov et al. 1998) In addition, small synthetic polyelectrolytes may induce domain separation in mixed membranes (Macdonald et al. 2000). In contrast to the short PLLs, for the sample with PLL 906 domain formation is much more obvious (Fig. 4b), as the peaks are much broader and resolved into different components. This is consistent

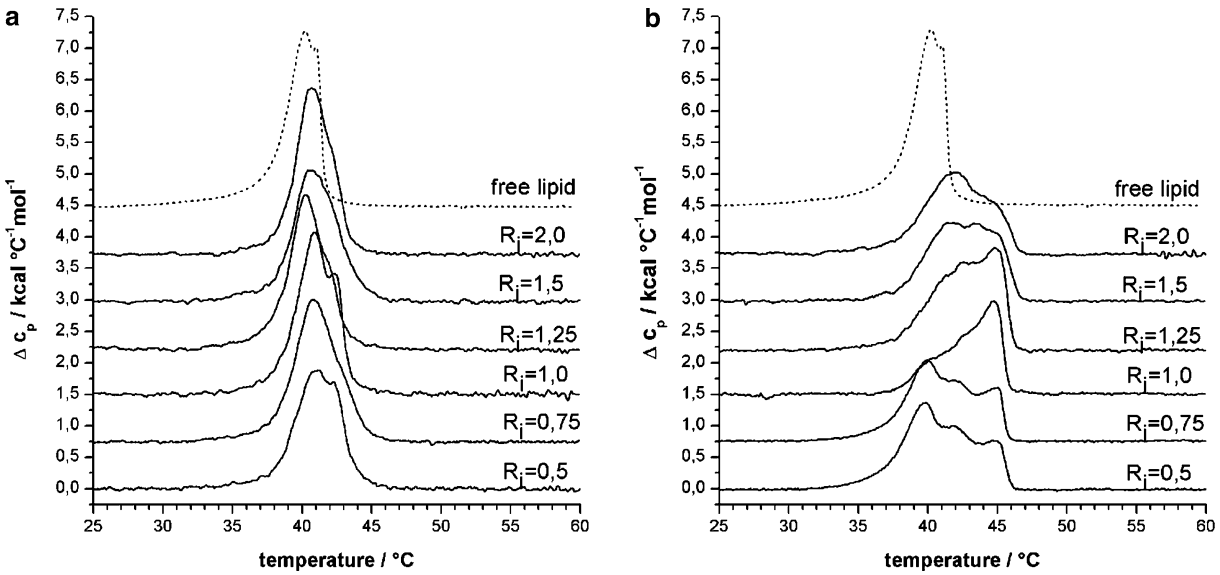

Fig. 4 DSC plots of the complexes of DPPG/DPPC (1/1, mol/mol) and PLL 14 (**a**) or PLL 906 (**b**) in different mixing rations R_i

with the chain length dependence of domain formation mentioned above. In addition, we observed a clear influence of the peak shapes on R_i. The transition peak consists of three components, which was also observed in the binary DPPG/PLL complexes. For the charge-neutralized complex ($R_i = 1$) the three components are less separated than in the case of lipid or peptide excess concentrations. The reason is probably the uniform coverage of the membrane surface. In case of peptide excess ($R_i = 0.5$), the low-temperature component has the highest intensity, whereas for $R_i = 1$ it is the high-temperature component, and for lipid excess ($R_i > 1$) the intermediate one. The complex with $R_i = 1$ is the most favourable one and thus its transition temperature is the highest. For the complexes of $R_i > 1$ not all lipids are bound and consequently T_m is lower. Complexes formed with peptide excess are unfavourable because of steric reasons and electrostatic repulsion between the excess positive charges. The long polypeptide chains are probably only partially bound, the positively charged ends and loops extend into the solution and prevent further binding of other polypeptide molecules. Thus, an optimal charge compensation cannot occur and the transition temperature is not as much increased as in the case of optimal binding and charge compensation.

FT-IR studies

To obtain more detailed information on which structures of the membrane are altered by PLL absorption and whether the secondary structure of the PLL changes upon binding we studied the complexes of PLL with liposomes by FT-IR as a function of temperature, membrane composition and peptide chain length. These temperature-dependent studies by FT-IR were compared to the results of the DSC experiments. Figure 5 shows an example of an FT-IR spectrum of a DPPG/PLL complex with the characteristic vibrational bands at two different temperatures, below and above the phase transition temperature.

DPPG/PLL complexes

The CH_2 stretching bands

The frequencies of the CH_2 stretching vibrations ($\nu_{as}(CH_2)$, $\nu_s(CH_2)$) reflect the order of the acyl chains in the hydrophobic region of the membrane. Highly ordered acyl chains with all *trans* conformation as observed in the gel phase lead to lower vibrational frequency. With increasing fractions of gauche isomers and decreasing van der Waals attractions in the liquid–crystalline phase the absorption maxima of the stretching bands will be shifted to higher frequency (Tamm and Tatulian 1997). In Fig. 6 the frequency of the symmetric CH_2 stretching vibration ($\nu_s(CH_2)$) of pure and complexed DPPG is plotted against temperature. The transition from gel to liquid–crystalline phase is clearly visible from the increase in frequency. The transition temperature determined by FT-IR compares well with the DSC results. Again we find an increase of T_m upon PLL binding, which is more pronounced for the longer polypeptide chains. As observed before in the DSC we see indications for domain formation in the samples prepared with the longer PLL (402 and 906), where the

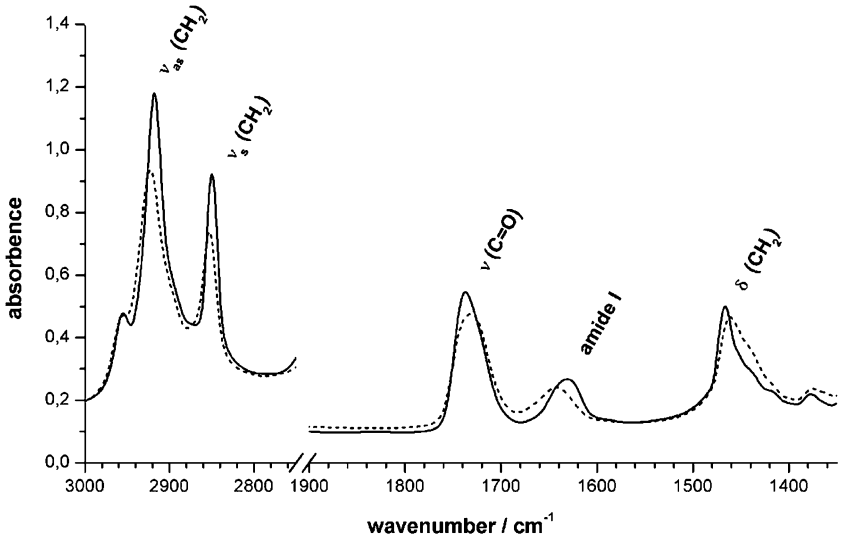

Fig. 5 FT-IR spectra of the complexes of DPPG with PLL 906 at 20°C (*solid line*) and 74°C (*dotted line*)

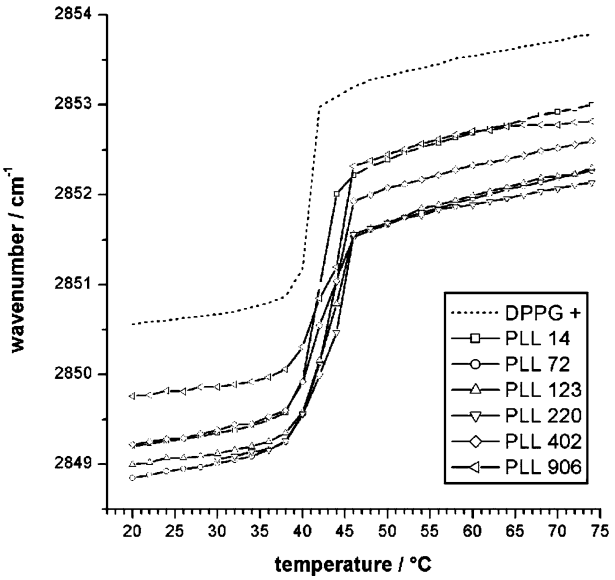

Fig. 6 Wavenumber of the maximum of the $\nu_s(CH_2)$ vibrational band in complexes of DPPG with PLL of different chain lengths. The lipid-to-peptide mixing ratio $R_i = 1$

traces show a two-step transition. We conclude that even though concentrations in the DSC and the IR experiments are different, identical results are obtained meaning that a change in total concentration has no measurable effect on the system behaviour.

The CH_2 stretching vibrations of the DPPG/PLL complexes are shifted to lower wavenumber in both gel and the liquid–crystalline phases. This indicates that the binding of PLL probably induces a higher order in the hydrophobic part of the membrane. The screening of the negative membrane charges by the bound peptide allows a better packing of the lipid molecules. The increase in conformational order of the acyl chains and the increased intermolecular vibrational coupling of the methylene stretching vibrations are also discussed by Carrier and Pézolet (1984) who compared Raman intensities. These effects might be due to a decreased tilt angle as was stated by Takahashi et al. (1992), which would enhance the van der Waals contact area.

The extent of the wavenumber shift depends again on the PLL chain length. Intermediate length PLL (PLL 72–220) causes the largest downshift in vibrational frequencies, whereas shorter and longer PLLs cause a smaller shift. Interestingly, the smallest shift is caused by the longest PLL 906. T_m and the wavelength of the absorption maxima do not show the same chain length dependence (Fig. 9b). Neither the absolute wavenumber nor the wavenumber shift between the gel and liquid–crystalline phase is directly correlated with T_m. Obviously, the increased order in the hydrophobic region is not the only factor that influences T_m.

The low wavenumbers for the CH_2 stretching bands are only reached after one heating and cooling cycle of the whole system. After addition of PLL to a DPPG membrane at room temperature only a slight change in the spectrum will be observed. Only when the membrane has passed into the liquid–crystalline phase the system is able to organize in an energetically favourable structure. The presence of a meta-stable phase before the first heating was also observed in the DSC experiments.

The lipid C=O band

Characteristic vibrational bands of the head group region are the carbonyl stretching vibration (ν_{CO}) and the phosphodiester band. The wavenumber of these bands is influenced by hydrogen bonding to water or to

other hydrogen bond donor groups. For stronger hydrogen bonds and/or more hydrogen bond donors the vibrational frequency of the lipid ester C=O group will be shifted to lower wavenumber (Blume et al. 1988). Therefore the C=O stretching band is a good indicator for the hydration of a membrane in the headgroup region. Actually, the observed band profile is due to at least two underlying bands separated by ca. 15 cm^{-1} originating from non-hydrated and hydrated C=O groups (Blume et al. 1988). In Fig. 7 the position of the absorption maximum is plotted against temperature. At the phase transition the wavenumber of the band is downshifted because the intensity of the lower frequency C=O band increases, i.e. the membrane is better hydrated in the liquid–crystalline phase than in the gel phase. Comparing the lipid/PLL complexes with the free DPPG membrane, we observe a small downshift in C=O band frequency upon PLL addition. The chain length dependence is less pronounced than for the CH_2 vibrations, but follows the same tendency. By PLL adsorption, the available space for hydration water will be reduced, as complexes with stacked bilayers are formed that are bridged by PLL. The water of hydration will be shared by bound PLL and the lipid headgroups. The lower frequency of the C=O band for the complexes indicates that the water molecules form slightly stronger and better directed hydrogen bonds with the lipid carbonyl groups.

The phosphate vibrations are not visible in these measurements because they are overlaid by the D_2O deformational band. Carrier and Pézolet (1986) report that the PO_2^- bands remain nearly unchanged.

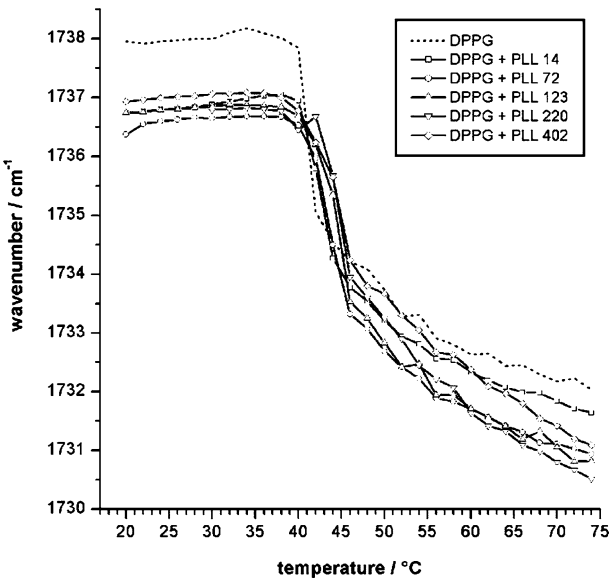

Fig. 7 Wavenumber of the maximum of the ν(CO) vibrational band in complexes of DPPG with PLL of different chain lengths. The lipid-to-peptide charge ratio $R_i = 1$

The peptide amide-I band

Analysis of the amide-I absorption band gives us information about the secondary structure of the peptide. PLL has the ability to adopt three common secondary structures: the α-helix, β-sheet and random coil (Greenfield et al. 1967). In neutral solution PLL forms a random coil. When the pH value is increased over 10.5, which is the pK value of the lysine side chain, it adopts an α-helix at low temperatures and a β-sheet after heating above 50°C (Carrier et al. 1990). The corresponding amide-I reference bands are given in Jackson et al. (1989) and reproduced by us. The β-sheet gives a sharp band with a maximum at 1,611 cm^{-1} and a less intense one at 1,680 cm^{-1}. The amide-I vibrations for the α-helix are found at 1,637 cm^{-1} and for the random coil structures at 1,644 cm^{-1}. Compared to proteins, these bands are found at unusually low wavenumbers. Jackson et al. (1989) assigned this peculiarity of PLL to a better vibrational coupling of the transitional dipole moments in a homopolypeptide and extremely high polar interactions with the solvent. The amide-I bands observed for the lipid/PLL complex are a superposition of the helix and the random coil component, which can be identified by calculating the second derivative spectrum. An example of an experimental amide-I band of a DPPG/PLL complex at two different temperatures is shown in Fig. 8. It is evident that the global band position of the amide-I band shifts to higher frequency at higher temperature. In the second derivative spectrum it can be seen that a band corresponding to an α-helix is evident at lower temperature and that at higher temperature only a band characteristic for a random coil is present. For the interpretation of the experiments at different temperatures we used, for simplification, the global maximum of the amide-I band. Its position is plotted in Fig. 9a as a function of temperature for different DPPG/PLL complexes. The band frequency is in the range typical for an α-helix or a random coil conformation. The shortest peptide PLL 14 always stays in a random coil structure. All longer peptides can form α-helices when bound to gel phase lipids with a remaining fraction being in random coil conformation. We observe a clear chain length dependence: the longer the polypeptide, the lower the wavelength of the amide-I band, i.e. the higher the proportion of α-helix. This can be explained by the higher binding constant of longer polypeptides. The analysis of the second derivative spectra reveals similar trends, namely that the low-frequency band at

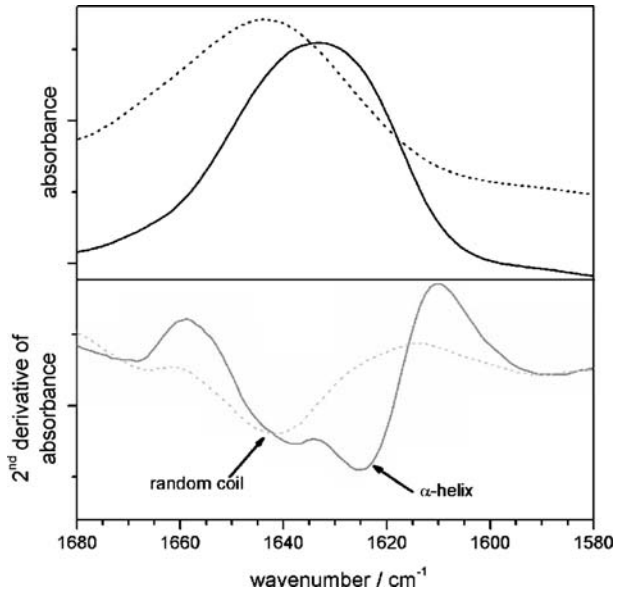

Fig. 8 Amide-I band of the complex DPPG/PLL 220 at 20°C (*solid line*) and 74°C (*dotted line*) and its second derivative spectra (in *grey*)

1,623 cm^{-1} characteristic for a bent α-helix (Jackson et al. 1989) becomes more intense at the expense of the component at 1,638 cm^{-1} when the PLL chain length is longer.

With increasing temperature the maximum of the amide-I band is shifted to higher wavelengths in all complexes, i.e. the fraction of random coil structure increases. This is apparently caused by a lowering of the charge density of the membrane at the phase transition and/or a concomitant desorption of PLL from the membrane surface. However, the peptide cannot be completely desorbed because we still observe a remarkable influence on the CH_2 and C=O stretching vibrations in the fluid–crystalline phase. Moreover preliminary results from isothermal titration calorimetric (ITC) experiments show that PLL binds to liquid–crystalline vesicles in a 1:1 stoichiometry (not shown). Therefore we assume that PLL in a random coil conformation remains bound to the membrane surface. Only for the complexes DPPG/PLL 72 and DPPG/PLL 123 is the α-helix to random coil transition partially coupled to the lipid phase transition as indicated by the larger wavenumber shift at T_m. For the longer peptides we just see a continuous increase in wavenumber with increasing slope over the whole temperature range. In no case a β-sheet is observed, as can be concluded from the lack of a low- and high-frequency components at about 1,611 and 1,680 cm^{-1}, respectively, in the second derivative spectra. Whereas in the bulk phase α-helices transform to β-sheets with rising temperature, the membrane seems to stabilize the helices at low temperature.

Similar results were also reported by Carrier and Pézolet (1984), who observed α-helices bound to DPPG in the gel phase by Raman spectroscopy. They also stated that short PLLs do not form α-helices on membrane surfaces (Carrier and Pézolet 1986). However, they studied only two different chain lengths of PLL and determined the structure only at 20°C. We now present for the first time information about the

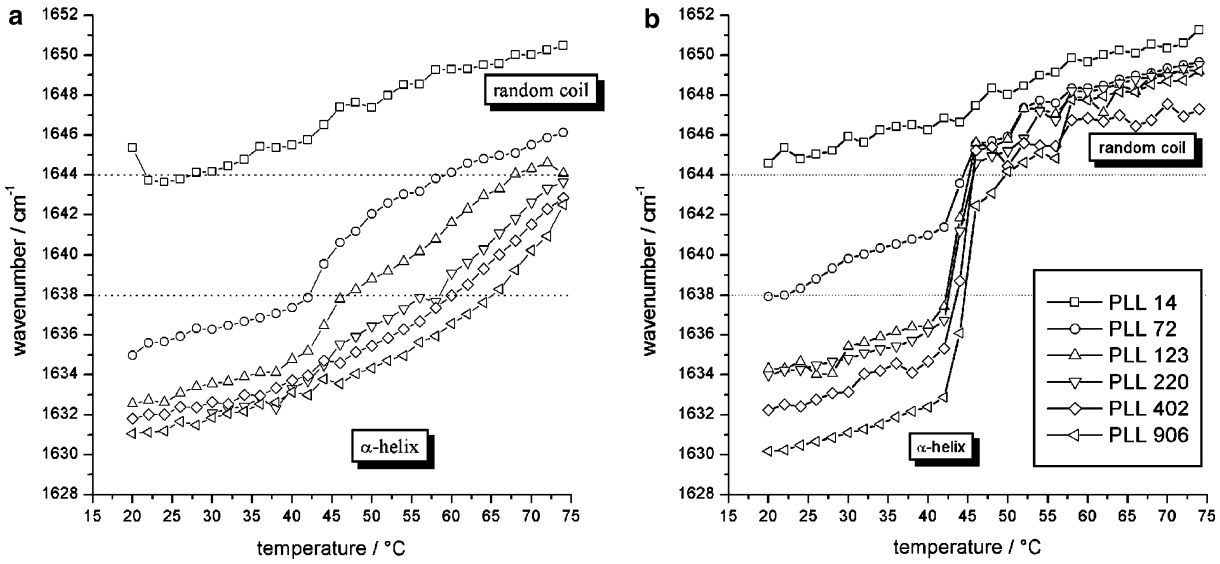

Fig. 9 Wavenumber of amide-I vibrational band maxima of PLL bound to pure DPPG membranes (**a**) and DPPC/DPPG mixed membranes (1/1, mol/mol) (**b**). The lipid-to-peptide charge ratio $R_i = 1$. The *dotted lines* indicate the typical frequencies for α-helical (1,638 cm^{-1}) and random coil (1,644 cm^{-1}) structures

complete thermotropic behaviour of PLL bound to DPPG membrane. In contrast to the results published by Carrier and Pézolet (1984) and our own results shown here, Fukushima et al. (1989) claimed that PLL binds in 50% α-helical and in 50% β-sheet structure to pure DMPG membranes as deduced from CD data. The drawback of this work is that the authors measured in the transition range of a complexed DMPG membrane at 25°C and at very low ionic strength. The lack of additional salt leads, on one hand, to an increased electrostatic interaction, but on the other hand it complicates the phase transition of DMPG even more (Schneider et al. 1999). Thus the membrane state was not well defined in their measurements and different peptide conformations might well arise from peptide being bound to different membrane structures.

Takahashi et al. (1996) observed a β-sheet conformation of PLL when bound to DPPA surfaces under experimental conditions that are comparable to ours. The reason for the formation of a different secondary structure on a negatively charged lipid membrane with different chemical structure is not clear at present, but could be due to the different headgroup sizes of the lipids and/or different degrees of hydration of the interface.

Interestingly, we find that the phase transition temperature T_m of DPPG is much better correlated with the secondary structure of the peptide than with the hydrophobic order or the headgroup hydration. PLL 14 that adopts only a random coil structure increases T_m only slightly, whereas all peptides that are bound as α-helices increase T_m much more and with the same chain length dependence, as found for the tendency for α-helix formation.

PLL complexes with mixed DPPG/DPPC membranes

We performed IR experiments also with mixed DPPG/DPPC membranes (1/1, mol/mol). In the spectral range of the CH_2 and the C=O vibrations all the tendencies for shifts in frequency are very similar to the pure DPPG system. However, the frequency shift of the amide-I band is remarkably different (Fig. 9b). In the gel state of the DPPG/DPPC membrane the secondary structure of the bound peptide is similar to the one we observed for pure DPPG, namely α-helical for all longer PLLs. But at T_m the wavenumber of the amide-I band suddenly increases strongly and shifts to values larger than 1,644 cm^{-1}. In the liquid–crystalline phase of the DPPG/DPPC membrane all peptides form almost exclusively random coils.

This behaviour could be explained by the reduced surface charge density in liquid–crystalline mixed DPPG/DPPC membranes. To form α-helices the positive charges of the lysine side chains need to be neutralized. In bulk solution this can be accomplished by a change in pH to values higher than the pK value of the side chain, i.e. a deprotonation of the terminal ammonium group of the side chain. When the peptide adsorbs to a pure DPPG membrane, the positive charges are neutralized by the negative membrane potential. After co-addition of neutral lipids, i.e. DPPC, the negative surface potential of the membrane might be too low to neutralize the lysine side chain charges, preventing the formation of a defined α-helical secondary structure. Therefore all PLLs will bind as random coil to an ideally mixed DPPG/DPPC membrane in the fluid state.

However, the peptide is bound as α-helix to gel-state DPPG/DPPC membranes. This indicates that PLL binding definitely must induce domain formation in the gel phase membrane. The DPPG molecules will move to the PLL binding sites, thus forming negatively charged domains that offer a high enough surface charge density for the PLL to form α-helical structure. These domains will coexist with DPPC-rich domains with lower surface charge density. Due to their higher lateral mobility in the fluid membrane state, the lipids will remix after passing the phase transition temperature. Consequently the regions of high negative surface charge disappear and a sudden change of the peptide secondary structure from α-helix to random coil is induced at the main transition temperature. This leads to a system, where a secondary structure change of a membrane-bound peptide is triggered by the phase transition of the membrane itself. The only results that point in the same direction were published by Laroche et al. (1988), who found that short PLL (ca. 20 monomers) being bound to DPPA membranes undergoes β-sheet to random coil transition upon heating above the membrane phase transition.

The notion of domain formation in the gel phase of mixed membranes induced by bound peptides is supported by an additional FT-IR spectroscopic experiment. To be able to detect the phase transitions of hypothetic domains separately, we used binary lipid mixes, in which one component had perdeuterated acyl chains. This allows us to simultaneously observe the CH_2 and the CD_2 stretching vibrations that are due to the isotopic shift of the CD_2 vibrations to lower wavenumbers well separated in the spectra. These two bands reflect the behaviour of the DPPG and the perdeuterated DPPC component, respectively. If the lipids demix in the gel phase and organize in different domains of which one is bound to PLL, we should observe slightly different transition temperatures for the perdeuterated DPPC and the non-deuterated DPPG components. Similar experiments were performed to prove domain

formation on mixed LPS/DMPE and DMPA/DMPC membranes upon interaction with PLL by Lasch et al. (1998) and Laroche et al. (1988). Our results are shown in Fig. 10. We see that the two lipids undergo their main transition at two slightly different temperatures, indicating that they are organized in separate domains. The second derivatives of the wavenumber plots presented in Fig. 10b reveal that a minor fraction of either lipid still contributes to the transition of the other component. This signifies that the lipids are not completely separated, but that a small quantity of DPPC is present in the DPPG domains and vice versa. The relative intensity changes observed in the plots in Fig. 10 indicate that the DPPG-rich domains contain less DPPC than the DPPC-rich domains contain DPPG, i.e. the miscibility gap induced by the PLL is not symmetric.

The well-separated transition temperatures indicate that PLL is only bound to one domain, namely the DPPG-rich domain. The DPPC-rich domain undergoes its transition at the same temperature as in the absence of PLL. Due the lack of charges PLL will not interact with DPPC molecules. This was proven by DSC and FT-IR experiments with pure DPPC membranes (results not shown). However, the DPPG-rich domain undergo its transition at a temperature that was also found for pure DPPG membranes.

Summary

In this paper the interactions of the positively charged peptide PLL with negatively charged lipid membranes (DPPG, DPPG/DPPC) was investigated as a function of temperature and peptide chain length. We studied the membrane phase transition, the hydrophobic order of the membrane and the head group hydration as well as the secondary structure of the peptides. DSC experiments revealed that PLL binding increases the main phase transition temperature T_m of pure DPPG by about 1–5°C. The exact value of increase depends on the PLL chain length. In general longer PLLs increase T_m more than shorter ones; however, steric hindrance in the case of longer PLLs and highly charged membranes can counteract this trend. These results obtained from DSC were supported by FT-IR experiments. Furthermore, FT-IR experiments showed that the acyl chain order in the hydrophobic part of the membrane is increased upon PLL binding, as is concluded from the downshift of the $\nu_s(CH_2)$ vibrational band of about 1–1.5 cm^{-1}. This was found for both gel and liquid–crystalline phases. A downshift of the carbonyl stretching vibration (ν(CO)) shows that hydrogen bonds to the hydrating water molecules get stronger or better oriented. Water molecules get immobilized in the restricted volume between the bilayer surface and the bound PLL. Analysis of the amide-I vibrational band showed that long PLL forms an α-helix as soon as it is bound to a negatively charged membrane with high surface charge density (DPPG), whereas it is a random coil in bulk solutions. When the membrane surface charge is diluted by the coaddition of neutral lipids (DPPC, DMPC), the conformation of the bound peptide depends on the phase state of the lipid membrane. In mixed DPPG/DPPC membranes

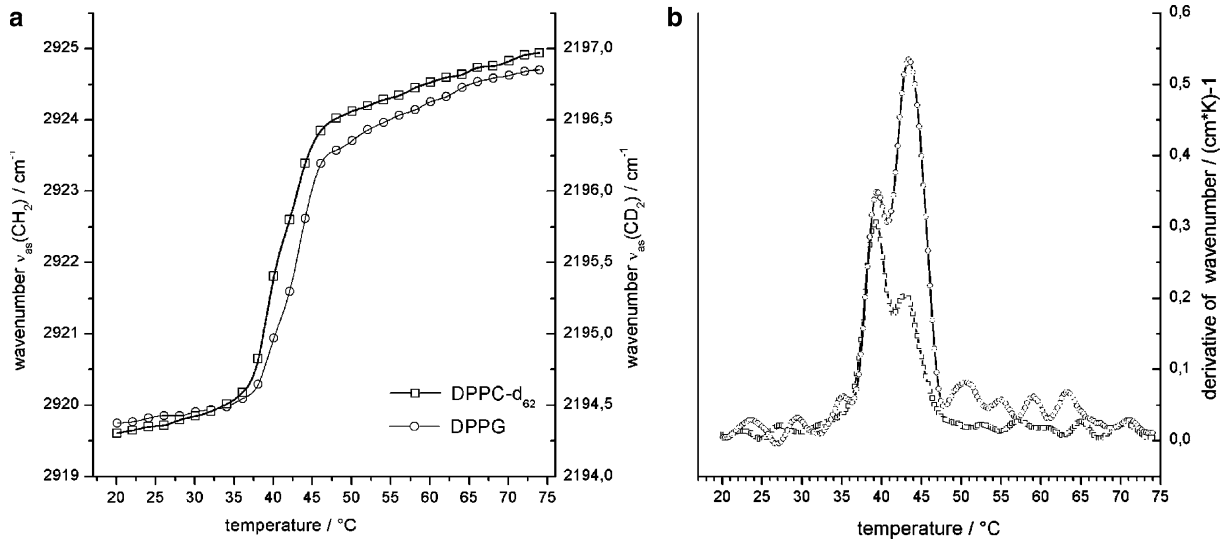

Fig. 10 **a** Temperature dependence of the antisymmetric methylene stretching vibrations of DPPG (*circles*) and deuterated DPPC (*squares*) in a equimolar mixed membrane, which is complexed with PLL 906, $R_i = 1$ (**a**) and its first derivatives (**b**). The derivative was taken after interpolating the data of **a** with a spline function. The peak maxima in **b** indicate the transition temperatures of either lipid species

PLL binding induces phase separation in the gel phase of the membrane and PLL is bound in α-helical conformation to the negatively charged DPPG-rich domains. Passing into the lipid crystalline phase the lipids remix and domain formation is reduced. This results in a α-helix to random coil transition of the peptide at the phase transition temperature of the mixed membrane. This is the first time that a phase transition-coupled highly cooperative change of peptide conformation was observed from α-helix to random coil for a number of longer chain PLLs.

Acknowledgment This work was supported by the Fonds der Chemischen Industrie.

References

Ben-Tal N, Honig B, Peitzsch RM, Denisow G, McLaughlin S (1996) Binding of small basic peptides to membranes containing acidic lipids: theoretical models and experimental results. Biophys J 71:561–575

Ben-Tal N, Honig B, Bagdasarian CK, Ben-Schaul A (2000) Association entropy in adsorption processes. Biophys J 79:1180–1187

Blondelle SE, Lohner K, Aguilar M-I (1999) Lipid-induced conformation and lipid-binding properties of cytolytic and antimicrobial peptides: determination and biological specificity. Biochim Biophys Acta 1462(1–2):89–108

Blume A, Hübner W, Messner G (1988) Fourier transform infrared spectroscopy of ^{13}C=O-labeled phospholipids hydrogen bounding to carbonyl groups. Biochemistry 27:8239–8249

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–dipalmitoylphosphatidyl–glycerol interactions in model membranes. Biochemistry 25:4167–4174

Carrier D, Dufourcq J, Faucon JF, Pézolet M (1985) A fluorescence 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

De Kruijff B, Cullis PR (1980) The influence of poly(L-lysine) on phospholipid polymorphism. Evidence that electrostatic polypeptide–phospholipid interactions can modulate bilayer/non-bilayer transitions. Biochim Biophys Acta 601:235–240

Denisov G, Wanaski S, Luan P, Glaser M, McLaughlin S (1998) Binding of basic peptides to membranes produces lateral domains enriched in the acidic lipids phosphatidylinositol 4,5-biphosphate: an electrostatic model and experimental results. Biophys J 74:731–744

Diederich A, Baehr G, Winterhalter M (1998) Influence of polylysine on the rupture of negatively charged membranes. Langmuir 14(16):4597–4605

Dolowy K (1979) Effect of interfacial tension and curvature of charged lipid bilayer–polylysine complexes. Bioelectrochem Bioenerg 6(2):305–307

Förster G, Schwieger C, Faber F, Weber T, Blume A (2006) Influence of poly-L-lysines on the structure of dipalmitoylphosphatidylglycerol/water dispersions studied by X-ray scattering. Eur Biophys J (this issue)

Franzin CM, Macdonald PM (2001) Polylysine-induced ^{2}H NMR-observable domains in phosphatidylserine/phosphatidylcholine lipid bilayers. Biophys J 81:3346–3362

Fukushima K, Muraoka Y, Inoue T, Shimozawa R (1988) Conformational change of poly(L-lysine) induced by lipid vesicles of dilauroylphosphatidic acid. Biophys Chem 30:237–244

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

Gad AE, Bental M, Elyashiv G, Weinberg H, Nir S (1985) Promotion and inhibition of vesicle fusion by polylysine. Biochemistry 24:6277–6282

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, Blume A (1999) Interaction of alkaline earth cations with the negatively charged phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol: a differential scanning and isothermal titration calorimetric study. Langmuir 15(17):5526–5534

Garidel P, Johann C, Mennicke L, Blume A (1997) The mixing behaviour of pseudobinary phosphatidylcholine–phosphatidylglycerol mixtures as a function of pH and chain length. Eur Biophys J 26:447–459

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

Hammes GG, Schullery SE (1979) Structure of macromolecular aggregates. II. Construction of model membranes from phospholipids and polypeptides. Biochemistry 9:2555–2563

Hartmann W, Galla HJ (1978) Binding of polylysine to charged bilayer membranes. Molecular organization of a lipid.peptide complex. Biochim Biophys Acta 509(3):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

Heimburg T (2000) A model for the lipid pretransition: coupling of ripple formation with the chain-melting transition. Biophys J 78:1154–1165

Heimburg T, Angerstein B, Marsh D (1999) Binding of peripheral proteins to lipid membranes: effect of lipid demixing upon binding. Biophys J 76:2575–2586

Jackson M, Haris PI, Chapman D (1989) Conformational transitions in poly(L-lysine): studies using Fourier transform infrared spectroscopy. Biochim Biophys Acta 998:75–79

Kim J, Mosior M, Chung LA, Wu H, McLaughlin S (1991) Binding of peptides with basic residues to membranes containing acidic phospholipids. Biophys J 60(1):135–148

Laroche C, Carrier D, Pézolet M (1988) Study of the effect of poly(L-lysine) on phosphatidic acid and phosphatidylcholine/phosphatidic acid bilayers by Raman spectroscopy. Biochemistry 27:6220–6228

Lasch P, Schultz CP, Naumann D (1998) The influence of poly (L-lysine) and porin on the domain structure of mixed vesicles composed of lipopolysaccaride and phospholipid: an infrared spectroscopic study. Biophys J 75:840–852

Lehrmann R, Seelig J (1994) Adsorption of Ca^{2+} and La^{3+} to bilayer membranes: measurement of the adsorption enthalpy and binding constant with titration calorimetry. Biochim biophys acta 1189(1):89–95

Loura LMS, Coutinho A, Silva A, Fedorov A, Prieto M (2006) Structural effects of a basic peptide on the organization of dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylserine membranes: a fluorescent resonance energy transfer study. J Phys Chem B 110:8130–8141

Macdonald PM, Crowell KJ, Franzin CM, Mitrakos P, Semchyschyn DJ (1998) Polyelectrolyte-induced domains in lipid bilayer membranes: the deuterium NMR perspective. Biochem Cell Biol 76:452–464

Macdonald PM, Crowell KJ, Franzin CM, Mitrakos P, Semchyschyn DJ (2000) ^{2}H NMR and polyelectrolyte-induced domains in lipid bilayers. Solid State Nucl Magn Reson 16:21–36

May S, Harries D, Ben-Shaul A (2000) Lipid demixing and protein–protein interactions in the adsorption of charged proteins on mixed membranes. Biophys J 79:1747–1760

Menger FM, Seredyuk VA, Yaroslavov AA (2002) Adhesive and anti-adhesive agents in giant vesicles. Angew Chem Int Ed 41(8):1350–1352

Menger FM, Seredyuk VA, Kitaeva MV, Yaroslavov AA, Melik-Nubarov NS (2003) Migration of poly-L-lysine through a lipid bilayer. J Am Chem Soc 125(10):2846–2847

Montal M (1999) Electrostatic attraction at the core of membrane fusion. FEBS Lett 447:129–130

Montich G, Scarlata S, McLaughlin S, Lehrmann R, Seelig J (1993) Thermodynamic characterisation of the association of small basic peptides with membranes containing acidic lipids. Biochim Biophys Acta 1146:17–24

Mosior M, McLaughlin S (1992) Binding of basic peptides to acidic lipids in membranes: effects of inserting alanine(s) between the basic residues. Biochemistry 31(6):1767–1773

Murray D, Arbuzova A, Hangyas-Mihalyne G, Gambhir A, Ben-Tal N, Honig B, McLaughlin S (1999) Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment. Biophys J 77(6):3176–3188

Novellani M, Santini R, Tadrist L (2000) Experimental study of the porosity of loose stacks of stiff cylindrical fibres: influence of the aspect ratio of fibres. Eur Phys J B 13:571–578

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

Roux M, Neumann JM, Bloom M, Devaux PF (1988) 2H and 31P NMR study of pentalysine interaction with headgroup deuterated phosphatidylcholine and phosphatidylserine. Eur Biophys J 16:267–273

Russ C, Heimburg T, Grünberg HH (2003) The effect of lipid demixing on the electrostatic interaction of planar membranes across a salt solution. Biophys J 84:3730–3742

Schneider MF, Marsh D, Jahn W, Kloesgen B, Heimburg T (1999) Network formation of lipid membranes: triggering structural transitions by chain melting. Proc Natl Acad Sci 96:14312–14317

Shai Y (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim Biophys Acta 1462(1–2):55–70

Shibata A, Murata S, Ueno S, Liu S, Futaki S, Baba Y (2003) Synthetic copoly(Lys/Phe) and poly(Lys) translocate through lipid bilayer membranes. Biochim Biophys Acta 1616:147–155

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(1):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

Tamm L, Tatulian S (1997) Infrared spectroscopy of proteins and peptides in lipid bilayers. Q Rev Biophys 30:365–429

Wagner K, Harries D, May S, Kahl V, Rändler JO, Ben-Shaul A (2000) Direct evidence for counterion release upon cationic lipid–DNA condensation. Langmuir 16:303–306

Wang J, Gambhir A, McLaughlin S, Murray D (2004) A computational model for the electrostatic sequestration of PI(4,5)P2 by membrane-adsorbed basic peptides. Biophys J 86(4):1969–1986

Yaroslavova AA, Kuchenkovaa OY, Okunevaa IB, Melik-Nubarova NS, Kozlovaa NO, Lobyshevb VI, Mengerc FM, Kabanova VA (2003) Effect of polylysine on transformations and permeability of negative vesicular membranes. Biochim Biophys Acta 1611:44–54