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Influence of transmembrane peptides on bilayers of phosphatidylcholines with different acyl chain lengths studied by solid-state NMR

Tomas Byström [a,*], Erik Strandberg [a], Frank A. Kovacs [b], Timothy A. Cross [b], Göran Lindblom [a]

[a] *Department of Chemistry, Biophysical Chemistry, Umeå University, SE-901 87 Umeå, Sweden*

[b] *Center for Interdisciplinary Magnetic Resonance at the National High Magnetic Field Laboratory, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL 32306-4005, USA*

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Abstract

The molecular orientation in a lipid membrane of the peptide fragment VEYAGIALFFVAAVLTLWSMLQYLSAAR (phosphatidylglycerophosphate synthase (Pgs) peptide E) of an integral membrane protein, Pgs, in *Escherichia coli* has been investigated by solid-state ^{15}N nuclear magnetic resonance (NMR) on macroscopically aligned lipid bilayers. The secondary structure of the peptide in lipid vesicles was determined by circular dichroism spectroscopy. Furthermore, the phase behaviour of the Pgs peptide E/dierucoylphosphatidylcholine (DEruPC)/water system was determined by ^{2}H, ^{31}P and ^{15}N solid-state NMR spectroscopy. The phase behaviour obtained was then compared to that of the Pgs peptide E solubilised in dioleoylphosphatidylcholine and water that was previously studied by Morein et al. [Biophys. J. 73 (1997) 3078–3088]. This was aimed to answer the question whether a difference in the length of the hydrophobic part of this peptide and the hydrophobic thickness of the lipid bilayer (hydrophobic mismatch) will affect the phase behaviour. The peptide mostly has a transmembrane orientation and is in an α-helical conformation. An isotropic phase is formed in DEruPC with high peptide content (peptide/lipid molar ratio (p/l) ≥ 1:15) and high water content (≥ 50%, w/w) at 35°C. At 55 and 65°C an isotropic phase is induced at high water content (≥ 50%, w/w) at all peptide contents studied (no isotropic phase forms in the lipid/water system under the conditions in this study). At high peptide contents (p/l ≥ 1:15) an isotropic phase forms at 20 and 40% (w/w) of water at 55 and 65°C. A comparison of the phase behaviour of the two homologous lipid systems reveals striking similarities, although the thicknesses of the two lipid bilayers differ by 7 Å. This suggests that the rationalisation of the phase behaviour in terms of the hydrophobic mismatch is not applicable to these systems. The C-terminus of Pgs peptide E is amphiphilic and a considerable part of the peptide is situated outside the hydrophobic part of the bilayer, a property of the peptide that to a large extent will affect the lipid/peptide phase behaviour.

Keywords: Lipid/peptide phase diagram; Membrane protein; Hydrophobic peptide; Nuclear magnetic resonance; ^{15}N chemical shift

Abbreviations: CD, circular dichroism; DEruPC, dierucoylphosphatidylcholine, di-22:1_c-phosphatidylcholine; DOPC, dioleoylphosphatidylcholine, di-18:1_c-phosphatidylcholine; H_{II}, reversed hexagonal liquid crystalline; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulphonic acid; L_α, lamellar liquid crystalline; NMR, nuclear magnetic resonance; p/l, peptide/lipid molar ratio; PC, phosphatidylcholine lipids; Pgs, phosphatidylglycerophosphate synthase from *Escherichia coli*; Pgs peptide E, VEYAGIALFFVAA-(^{15}N)VTLWSMLQYLSAAR; SAXS, small angle X-ray scattering; TFE, 2,2,2-trifluoroethanol; WALP, HCOAWW(LA)$_n$WWAN-CH_2CH_2OH

* Corresponding author. Fax: +46-90-786-7779; E-mail: tomas.bystrom@chem.umu.se

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1. Introduction

Interactions between lipids and proteins in membranes are crucial for many of the processes occurring in biological cells, e.g. lipid-dependent enzyme activities or the folding of integral membrane proteins ([1] and references cited therein). To get a better understanding of such interactions we perform solid-state nuclear magnetic resonance (NMR) investigations of various transmembrane peptides in different lipid systems. The intention of this work is two-fold, namely (a) to study the orientation in a lipid bilayer of a hydrophobic peptide having an appropriate NMR label, and (b) to investigate the effect of transmembrane peptides on the phase structure of phosphatidylcholines (PCs) with acyl chains of different chain lengths. With these purposes in mind we have investigated a putative transmembrane peptide, which is a fragment of a membrane protein involved in the biosynthesis of lipids in *Escherichia coli*.

The integral membrane-bound protein phosphatidylglycerophosphate synthase (Pgs) takes part in the biosynthetic pathway for phospholipids in *E. coli*. It is a rather small protein with 182 amino acid residues and a molecular weight of 20.7 kDa [2] and it is tightly associated with the cytoplasmic membrane [3]. One of the products of the Pgs enzyme is phosphatidylglycerophosphate which in a later step is transformed to 1,2-diacyl-*sn*-glycero-3-phosphoglycerol lipids that constitute 20–25% of the lipids in *E. coli*. [4]. Pgs was scanned for probable membrane-spanning α-helical segments, and one of the five predicted membrane-spanning α-helical segments, called Pgs peptide E (amino acid residues 149–176, VEYAGIALFFVAAVLTLWSMLQYLSAAR, in Pgs), has been synthesised. The peptide solubilised in sodium dodecyl sulphate micelles has been shown by high-resolution NMR techniques to adopt an α-helical conformation [5], which was also confirmed by circular dichroism (CD) spectroscopy on the peptide incorporated in dioleoylphosphatidylcholine (DOPC) vesicles [6].

For an investigation of lipid/peptide interactions, it is a prerequisite to know how the peptide is oriented relative to the lipids. By ^{15}N labeling the amide nitrogen of one amino acid residue in the peptide, the orientation of α-helical peptides can be measured by ^{15}N solid-state NMR techniques [7–10]. Therefore, samples of a single lamellar liquid crystalline (L_α) phase have been prepared with a ^{15}N-labeled peptide, and ^{15}N solid-state NMR has been used to characterise the peptide orientation and dynamics.

It has been found that some transmembrane peptides induce both reversed hexagonal liquid crystalline (H_{II}) and isotropic phases with membrane lipids [6,11,12]. For gramicidin A [11] and HCOAWW-$(LA)_n$WWANCH_2CH_2OH (WALP) peptides [12] it has been shown that the difference between the hydrophobic thickness of the lipid bilayer and the length of the hydrophobic part of the peptide plays an important role in determining the phase structure. This study is aimed at gaining further information on changes in the phase behaviour of a system where the Pgs peptide E is incorporated into phosphatidylcholine lipids with unsaturated acyl chains of different lengths. Previously, a tentative phase diagram for the Pgs peptide E/DOPC/water system has been determined [6]. Here, we have extended that study to include a bilayer composed of dierucoylphosphatidylcholine (DEruPC), which has acyl chains with 22 carbon atoms, i.e. the lipid bilayer thickness is 7 Å larger [13] for this system than for the DOPC system.

2. Materials and methods

2.1. Materials

The Pgs peptide E was synthesised by Dr Åke Engström, at the Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden. The amide nitrogen of the alanine-13 residue in the peptide was labeled with ^{15}N. The purity of the peptides was checked with plasma desorption mass spectrometry and amino acid composition analysis, and it was used without further purification.

DEruPC and DOPC lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and were used without further purification after checking the purity with thin layer chromatography analysis.

Deuterium oxide ^2H_2O (99.9%) was purchased from Larodan Fine Chemicals (Malmö, Sweden). Trifluoroacetic acid was purchased from Merck (Darmstadt, Germany) and 2,2,2-trifluoroethanol

(TFE) from Sigma (St. Louis, MO, USA). The water was either deionised and filtered with a Milli-Q Water purification system (Millipore, Bedford, MA, USA) or high performance liquid chromatography grade water purchased from Fischer Scientific (Pittsburgh, PA, USA).

2.2. Sample preparation

The protocol for sample preparation closely follows that described in [6]. Some of the samples were prepared without the two-step dissolution of the peptide, and in these cases the peptide was directly dissolved in TFE. After that, the steps were identical to those in the original protocol.

A third protocol was also used where the lipids were suspended in water and sonicated for 1.5–2 h using an MSE Soniprep 150 sonifier to yield unilamellar vesicles. Titanium particles were removed by centrifuging the samples for 15 min at 4500 rpm in a swing-out rotor in a Hettich Universal 30 F centrifuge. Then peptide dissolved in TFE was added drop by drop to the vesicle solution under vigorous vortexing. The resulting solution was then ultrafiltrated using a Centricon-10 tube (Millipore, Bedford, MA, USA) to yield a concentrated solution which was lyophilised. The lyophilised material was transferred to 8 mm glass sample tubes and dried under high vacuum. The samples were then hydrated with 10 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulphonic acid (HEPES), pH 7.0 in ^2H_2O and the tubes were immediately flame-sealed. Samples were homogenised by centrifuging them back and forth in the sealed tubes and they were then left in a dark place at room temperature for at least a week before NMR measurements.

NMR measurements showed that the differences between samples prepared with the modified protocols and the original protocol were negligible (data not shown).

Samples for CD measurements were prepared mostly the same way as the NMR samples. After the lyophilisation the dried material was suspended in approximately 2 ml of buffer (10 mM HEPES, pH 7.0) and sonicated for 40 min with an MSE Soniprep 150 sonifier at an effect of 8 mW. The sample tube was immersed in a water bath during the sonication. Titanium particles were removed by centrifugation for 20 min in a swing-out rotor at 4700 rpm in a Hettich Universal 30 F centrifuge.

The hydrated powder sample for ^{15}N NMR was prepared with a water content of 25% (w/w), and the peptide/lipid molar ratio (p/l) was 1:7, which is the point in the phase diagram with the highest peptide concentration that is safely in the single L_α phase region even at high temperatures [6].

Oriented samples were prepared according to the method of Kovacs and Cross [21]. A p/l of 1:16 was used with 50% (w/w) water. This is on the border between the L_α and isotropic phases in the phase diagram. However, the orientation procedure with glass plates favours the L_α phase, and according to ^{31}P NMR spectra no isotropic phase was formed. 85 mg DOPC and 20 mg Pgs peptide E were solubilised in 500 μl TFE and spread in a thin layer on 46 glass plates, 75 μm thick (Marienfeld Laboratory Glassware, Bad Mergentheim, Germany) and with an area of 5.8×15 mm^2. The plates were allowed to dry in air for 12 h and in vacuum for 24 h. The dry plates were stacked in a square borosilicate sample tube, of 6×6 mm inner dimensions, that was flame-sealed at one end. High performance liquid chromatography grade water was added to 50% (w/w) hydration. The sample tube was sealed with a square glass cover and epoxy glue before incubating at 50°C for approximately 2 weeks.

2.3. NMR spectroscopy

2.3.1. Determination of phase diagrams

^2H NMR spectra were recorded in Umeå on a Chemagnetics Infinity spectrometer operating at a proton Larmor frequency of 100.04 MHz. Quadrupole splittings from ^2H_2O in the sample were measured at 15.36 MHz using a quadrupolar echo pulse sequence [14]. The ^2H 90° pulse width was 16 μs and typically 4000 scans were averaged with a relaxation delay of 1 s.

^{31}P NMR spectra were recorded in Umeå on a Chemagnetics Infinity spectrometer operating at a proton Larmor frequency of 400.52 MHz. The chemical shift anisotropy lineshapes were recorded at 162.13 MHz using a Hahn echo pulse sequence with extended phase cycling [15]. The ^{31}P 90° pulse

width was 18 μs and typically 2000 scans were averaged with a relaxation delay of 1.5 s. Protons were decoupled during data acquisition using the WALTZ decoupling scheme [16]. External H_3PO_4 was used as a chemical shift reference.

The sample temperature during the measurements was controlled by variable temperature units utilising an air stream around the sample to maintain the temperature. A thermistor was used to measure the temperature in the probe. The samples were allowed to equilibrate for at least 30 min at each temperature.

2.3.2. Determination of peptide orientation

^{31}P NMR spectra for aligned samples were recorded in Tallahassee, FL, USA, on a narrow bore IBM/Bruker 200SY spectrometer with a home-built solids package. A double resonance $^{31}P/^{1}H$ probe was used for proton decoupling. The ^{31}P experiments were performed at a resonance frequency of 80.99 MHz with a recycle delay of 4 s and a 90° pulse width of 11 μs. The ^{31}P spectra were referenced to the H_3PO_4 resonance in water assigned to zero ppm.

^{15}N NMR spectra were recorded in Tallahassee, FL, USA, on a home-built 400 MHz spectrometer using a Chemagnetics data acquisition system and a wide bore Oxford Instruments 400/89 magnet. A double frequency probe permitted proton decoupling at a field strength of 70 kHz and cross-polarisation. Spectra of hydrated samples were obtained at a resonance frequency of 40.6 MHz with a contact time of 1 ms, recycle delay of 4 s and a 90° pulse width of 4.5 μs. Spectra of the dry peptide powder were obtained with a 6 s recycle delay and a 5.5 μs 90° pulse. The ^{15}N spectra were referenced using $^{15}NH_4NO_3$ as an external chemical shift reference assigned to zero ppm. The NMR experiments were performed at room temperature.

All the NMR data were processed using the Spinsight software provided by Chemagnetics.

2.4. Small angle X-ray scattering (SAXS)

SAXS measurements were made using Station 8.2 at Daresbury Labs (Cheshire, UK). The wavelength of the beam was 1.54 Å and the camera length was 1.5 m. The samples were applied between mica sheets in a copper sample cell which was mounted on a Linkam temperature control stage. The samples were allowed to equilibrate at least 15 min at each temperature. Wet rat tail collagen was used as a reference for distance measurements. The data were processed using the xotoko software (Geoff Mant, Daresbury Labs).

2.5. CD spectroscopy

CD measurements of Pgs peptide E in DOPC and DEruPC vesicles were carried out on a JASCO 720 spectropolarimeter using cells with 1 mm pathlength, a bandwidth of 1 nm, a response time of 1 s and a scan speed of 20 nm/min. A baseline correction was achieved using spectra from control samples prepared without the peptide.

3. Results

3.1. Peptide secondary structure

The secondary structure of the peptide was investigated by CD measurements on peptides incorporated in sonicated DEruPC vesicles. Fig. 1 shows a typical CD spectrum recorded with a p/l of 1:10 at 25°C. This spectrum shows the characteristics typical for an α-helix: minima at 223 nm and near 208 nm (the latter is not clearly pronounced) and a crossover near 202 nm [17]. A change in the temperature between 25 and 55°C did not affect the lineshape significantly.

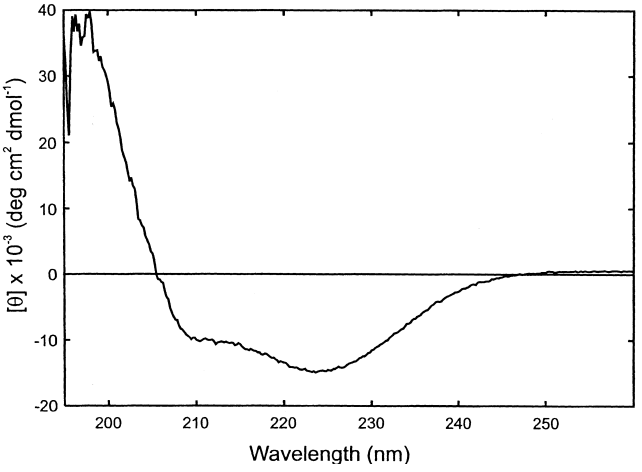

Fig. 1. CD spectrum of Pgs peptide E in DEruPC vesicles. The p/l was 1:10 and the temperature was 25°C.

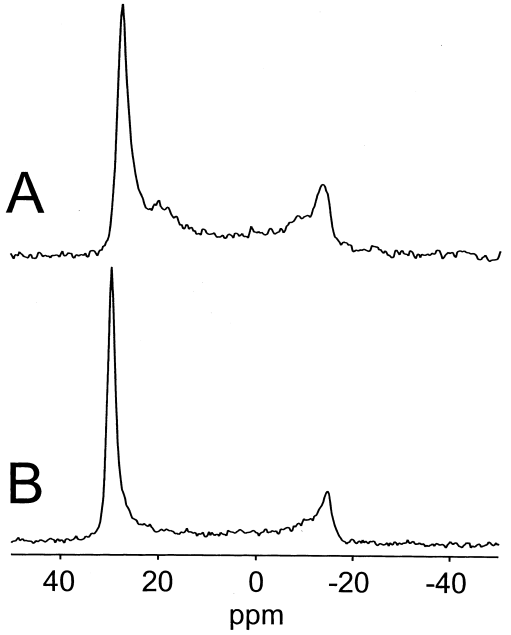

Fig. 2. ^{31}P NMR spectra of macroscopically oriented samples. (A) Pgs peptide E/DOPC/water sample, with p/l 1:16 and 50% (w/w) water. (B) DOPC/water sample, 50% (w/w) water.

3.2. Peptide orientation

The orientation of the peptide in DOPC bilayers was measured by ^{15}N NMR on labeled peptides in oriented samples. Unfortunately, it was not possible to get a sufficiently well oriented sample for NMR studies with DEruPC. The degree of orientation in the oriented samples was investigated by ^{31}P NMR. The orientation of the phospholipids can be estimated by integration to be about 50% oriented and 50% unoriented. This latter amount may reflect disorder about two or three axes. Fig. 2A shows the ^{31}P NMR spectrum of an oriented sample with a peptide to lipid ratio of 1:16 and 50% (w/w) water. Fig. 2B shows the corresponding spectrum of an oriented DOPC/water sample, 50% (w/w) water.

A ^{15}N NMR spectrum of the dry peptide powder was recorded, showing the chemical shift anisotropy tensor elements to be $\sigma_{11} = 37$ ppm, $\sigma_{22} = 58$ ppm, and $\sigma_{33} = 205$ ppm (Fig. 3A). Fig. 3B shows the ^{15}N NMR spectrum of the hydrated peptide powder sample with a peptide to lipid ratio of 1:7 and 20% (w/w) water. In this spectrum, the chemical shift anisotropy is reduced, indicating an axially symmetric chemical shift anisotropy tensor. Because of the low sensitivity of ^{15}N NMR and the limited amount of peptide, it was not possible to obtain a hydrated

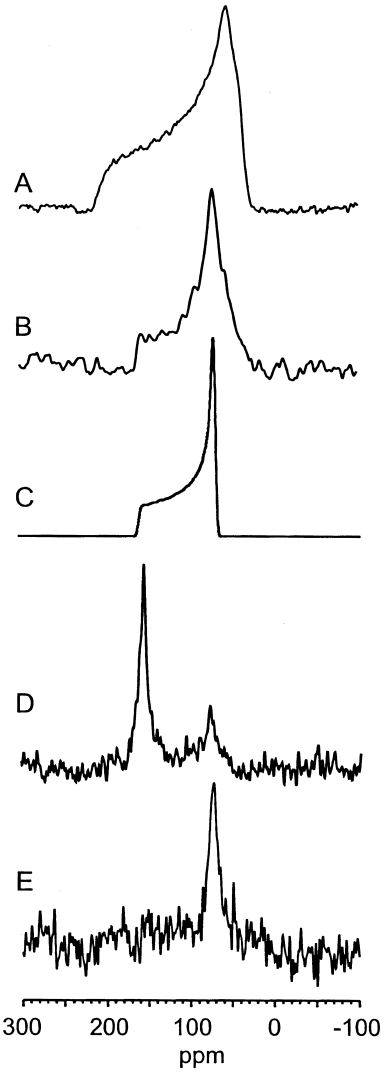

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Fig. 3. (A) ^{15}N NMR spectrum of the dry Pgs peptide E powder. Approximately 100 mg of ^{15}N-labeled Pgs peptide E was used, the signal was averaged over 26 896 scans, and a 40 Hz exponential line broadening was applied. (B) ^{15}N NMR spectrum at 39°C of a hydrated Pgs peptide E/DOPC/water powder sample, with p/l 1:7 and 20% (w/w) water. 27 mg of peptide, 19 168 scans, and 100 Hz exponential line broadening. (C). Simulated ^{15}N NMR spectrum fitted to the spectrum in (B). (D) ^{15}N NMR spectra of a macroscopically aligned hydrated Pgs peptide E/DOPC/water sample, with a p/l of 1:16 and 50% (w/w) water. 20 mg of Pgs peptide E was used. The bilayer normal parallel to the external magnetic field, 42 304 scans, and 40 Hz exponential line broadening. (E) As in (D), but with the bilayer normal perpendicular to the external magnetic field, 22 528 scans, and 40 Hz exponential line broadening.

powder spectrum with a very good signal to noise ratio. A simulated spectrum fitted to the experiment (Fig. 3C) shows that the chemical shift anisotropy tensor is axially symmetric with the elements $\sigma_{\parallel} = 160$ ppm, and $\sigma_{\perp} = 70$ ppm. Within the margin of error, this is consistent with the requirement that:

$$\sigma_{11} + \sigma_{22} + \sigma_{33} = \sigma_{\parallel} + 2\sigma_{\perp} \quad (1)$$

Considerable intensity between 30 and 70 ppm exists in the spectrum, which is, at least in part, due to the natural abundance of ^{15}N of the phosphocholine headgroup. The lack of intensity between 160 and 205 ppm indicates that there are no detectable unhydrated peptides and that the motional freedom of each molecule is similar throughout the sample.

When the macroscopically aligned peptide samples were oriented with the normal of the lamellar plane, parallel to the magnetic field, the ^{15}N NMR spectrum showed a peak at 157 ppm, and some powder pattern from unoriented parts of the sample (Fig. 3D). The resonance from the oriented sample has the chemical shift of $\sigma_{\parallel}$. When the sample was oriented with the lamellar plane normal, perpendicular to the magnetic field, a peak at 73 ppm was recorded (Fig. 3E) consistent with the $\sigma_{\perp}$ chemical shift. These shifts show that the helix is on average oriented parallel to the bilayer normal. If the helix had been tilted, the chemical shifts of the oriented samples would have been at intermediate values. The fact that the chemical shifts differ somewhat between the hydrated powder and the oriented samples may be due to the different compositions of the samples.

3.3. Phase diagrams

The phase behaviour of the Pgs peptide E/DEruPC/^2H_2O system was investigated using the ^{31}P and ^2H NMR methods described by [18]. Some SAXS measurements were also performed which corroborated the NMR measurements. A fair number of samples were prepared whose compositions are denoted by circles in the tentative phase diagrams shown in Fig. 4. The phase behaviour was studied at 35, 55 and 65°C. The phase behaviours at different temperatures were the same regardless of the thermal history of the samples, i.e. the samples exhibited the same spectra at 35°C before and after heating to 65°C. The phase behaviour is also reproducible

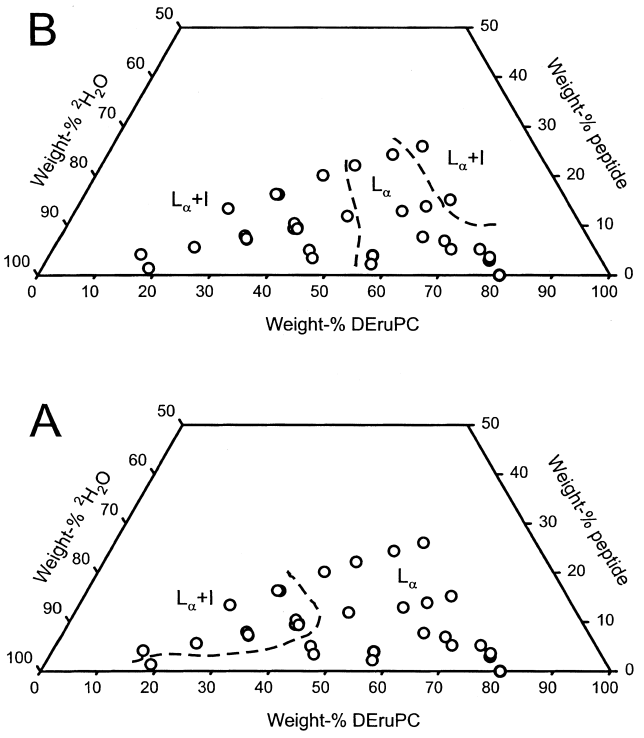

Fig. 4. Partial pseudo-ternary phase diagrams of the system consisting of Pgs peptide E/DEruPC/^2H_2O (10 mM HEPES pH 7.0). The samples examined are denoted by circles. The dashed lines indicate tentative phase boundaries. The phase diagrams were constructed from data recorded at (A) 35°C and (B) 55°C.

both by measuring several times on each sample (the samples were stable for at least a month) and for duplicate samples. It should be noted that we do not have a strict three-component system since we use a buffer system consisting of several components instead of pure water. However, we chose to represent the phase diagrams in triangular form for convenience (i.e. they are pseudo-ternary phase diagrams, see also [6,18]).

At 35°C (see Fig. 4A) the L_α phase is the dominating anisotropic phase in the phase diagram. It is only in samples with both high peptide (p/l $\geq$ 1:15) and water ($\geq$ 50%, w/w) content that some isotropic phase is in equilibrium with the L_α phase. At high water content (80%, w/w) and low peptide content (p/l = 1:50) an L_α phase is formed, and an isotropic phase in equilibrium with an L_α phase is formed at higher peptide content (p/l = 1:15). It is notable that the L_α phase is stable at such a high p/l as 1:7 over a rather broad range of water contents (20–$\sim$45%, w/w).

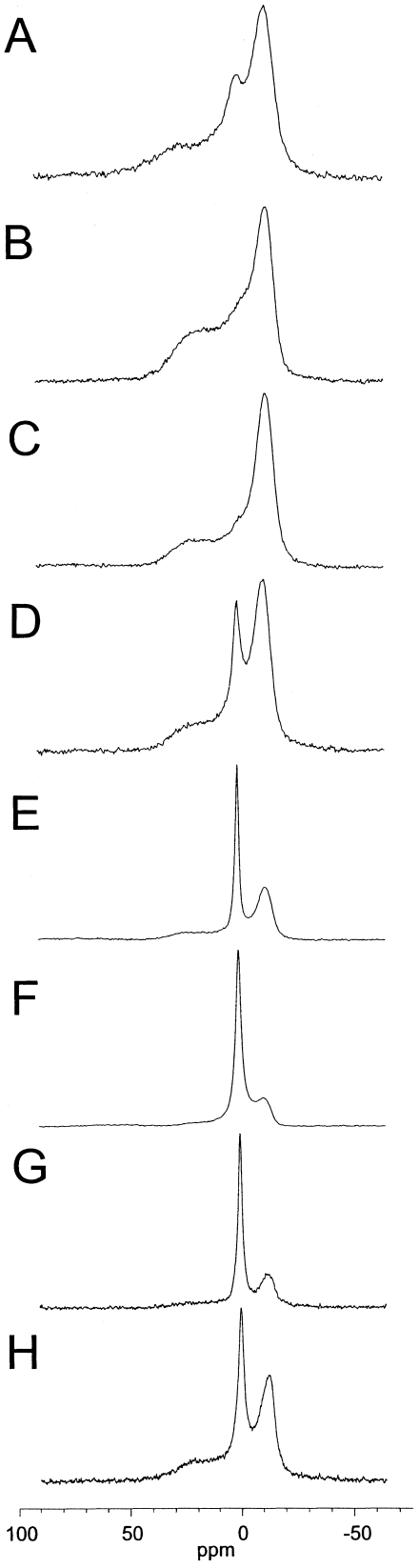

Fig. 5. Representative ^{31}P NMR spectra from samples with p/l of 1:15. The temperature is 55°C. The $^{2}H_2O$ (10 mM HEPES pH 7.0) contents are (top to bottom) 20% (A), 25% (B), 30% (C), 40% (D), 50% (E), 60% (F), 70% (G), and 80% (H) (w/w).
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At 55°C (see Fig. 4B) an isotropic phase in equilibrium with an L_α phase appears at low water content (20%, w/w) and high peptide content (p/l = 1:15 and 1:7) (examples of ^{31}P NMR spectra are shown in Fig. 5). The isotropic phase in equilibrium with an L_α phase in the high water content region starts to form at 40% (w/w) water (Fig. 5D–H). No single L_α phase is formed with ≥ 50% (w/w) water and regardless of composition no single isotropic phase is obtained.

There is no substantial difference between the phase behaviour at 55 and 65°C. However, an iso-

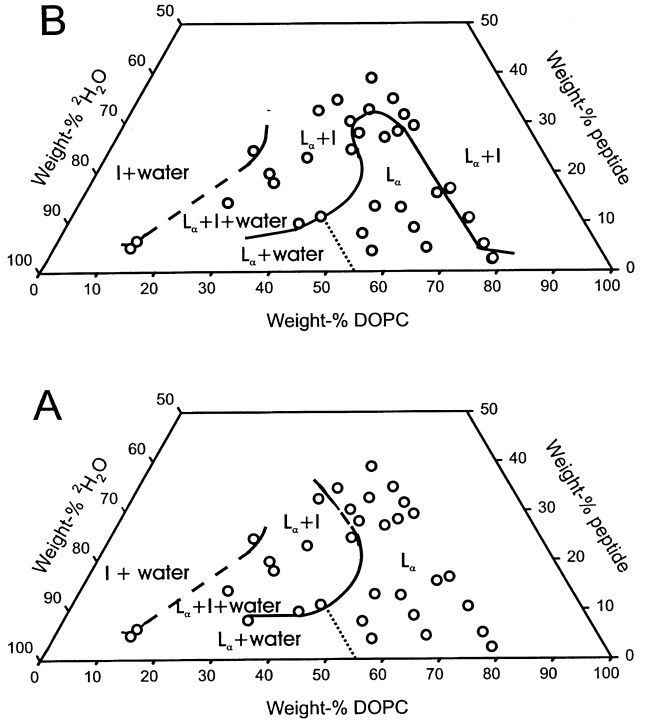

Fig. 6. Partial pseudo-ternary phase diagrams of the system consisting of Pgs peptide E/DOPC/$^{2}H_2O$ (10 mM HEPES pH 7.0). The samples examined are denoted by circles. The solid and dashed lines indicate firm and tentative phase borders, respectively. The dotted border lines denote the boundary of the L_α phase toward excess water. The phase diagrams were constructed from data recorded at (A) 25°C and (B) 55°C. (Adapted from [6].)

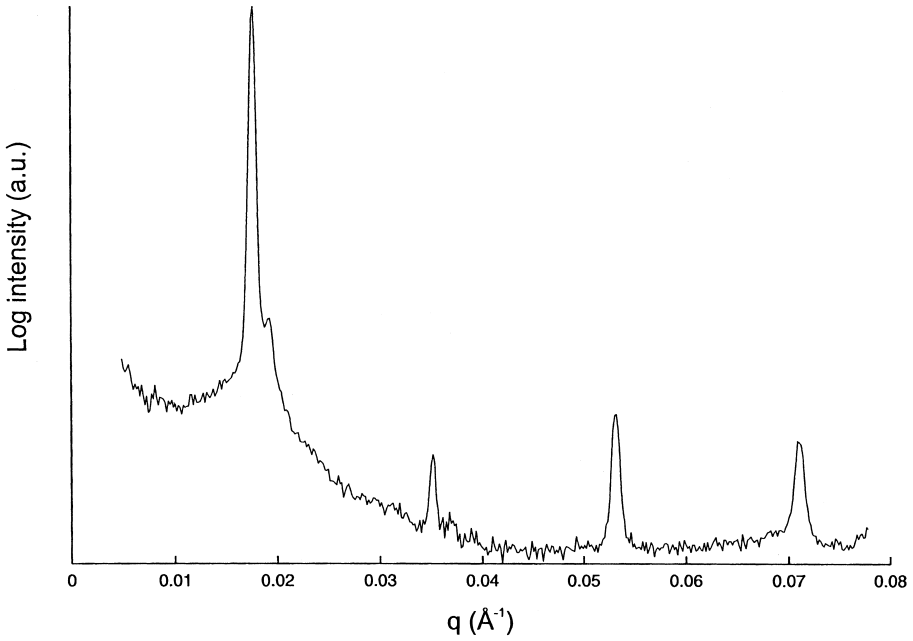

Fig. 7. X-ray diffraction pattern for a sample from the Pgs peptide E/DEruPC/^2H_2O (10 mM HEPES pH 7.0) system with a p/l of 1:15 and a water content of 25% (w/w).

tropic phase in equilibrium with an L_α phase is formed at 30% (w/w) water and p/l = 1:7 at 65°C, while at 55°C only an L_α phase is observed.

4. Discussion

From high-resolution NMR spectroscopy [5] and CD ([6] and this work) it is concluded that the Pgs peptide E adopts a mostly α-helical conformation. It is also indicated by the quite low absolute value of the ellipticities at 208 and 222 nm (Fig. 1) that a part (notably the ends) of the peptide has a random coil conformation. A precise determination of the orientation of the transmembrane peptide in lipid bilayers has not been determined previously. Therefore, we have in this work determined the orientation of the peptide in an L_α phase, and also studied the effect on the lipid phase structure upon solubilisation of an increasing concentration of the peptide in lipid bilayers composed of PCs with different acyl chain lengths.

4.1. Peptide orientation by solid-state ^{15}N NMR

A comparison of the dry powder pattern (Fig. 3A) and the hydrated powder pattern (Fig. 3B) shows a reduced chemical shift anisotropy of the peptide label in a lipid system. This indicates that the peptide is rotating fast around some axis close to the σ_{33} axis, averaging the chemical shift tensor elements and giving an axially symmetric tensor. If the peptide is lying on the bilayer surface with the more hydrophobic residues in the acyl chain region and the more hydrophilic residues closer to or in the aqueous environment, then fast rotation is unlikely as a rotation in this case would expose hydrophobic residues to the water and hydrophilic residues to the hydrophobic lipid acyl chains. Therefore, fast rotation indicates a transmembrane orientation of the peptide. The fast motion also indicates that the peptide is not in a large aggregate.

The macroscopically aligned sample with the bilayer normal parallel to the external magnetic field generates a resonance at $\sigma_{\parallel}$. This is a clear indication that the peptide is oriented with the motional axis parallel to the magnetic field spanning the lipid bilayer, and thus perpendicular to the lamellar planes.

The fact that the ^{15}N NMR peaks from an oriented L_α phase have the chemical shifts of $\sigma_{\parallel}$ and $\sigma_{\perp}$ shows that the tilt angle of the α-helix is on average equal to zero. This could be due to a tilted

helix, rotating around the bilayer normal at a rate that is fast compared to nuclear spin interaction magnitudes (or, more unlikely, the peptide long axis may rotate fast exactly perpendicular to the membrane plane).

Some powder pattern, originating from unoriented parts of the sample, is also seen. It can be estimated from simulations that this unoriented part contains less than 30% of the peptide. Part of this unoriented sample is probably located outside the glass plates, i.e. the sample consists partly of a powder. Another source for the unoriented sample is defects, such as oily streaks and parabolic focal conics [19]. When the aligned sample is turned, so that the bilayer normal is perpendicular to the external magnetic field, a single peak at $\sigma_{\perp}$ is obtained. This confirms that the peptide is rotating fast on the NMR timescale. If the peptide had performed a very slow rotation, or no rotation at all, a powder average generating a very broad lineshape would be observed.

The $\sigma_{\parallel}$ resonance is relatively sharp, with a half-height linewidth of about 9 ppm (360 Hz) without line broadening. The peaks are also symmetrical, seen most easily in Fig. 3D. This indicates that the peptides are well oriented with a small mosaic spread in the lipids and little heterogeneity in the peptide structure or in the peptide environment. If there were a broader distribution of orientations, the peaks would be asymmetric with one sharp edge depending on the intrinsic linewidth and one broader edge caused by an orientational range.

The fast rotation and the relatively small linewidths suggest that the Pgs peptide E is not highly aggregated in the DOPC bilayers. The linewidth is a little larger than for gramicidin A [20], but much less than for the influenza A M2 peptide [21]. One reason for the latter observation could be that, since the M2 is a four-helix bundle, it may possess significant conformational heterogeneity while the single transmembrane Pgs peptide E does not.

The ^{15}N NMR measurements show that the Pgs peptide E adopts a transmembrane orientation in DOPC bilayers, and it is, therefore, very likely that this situation pertains also to the peptide in DEruPC.

4.2. Determination of the phase diagrams

If one compares the tentative phase diagrams obtained in [6] for the Pgs peptide E/DOPC/^2H_2O system (see Fig. 6) with those obtained in this study (Pgs peptide E/DEruPC/^2H_2O, see Fig. 4) the similarities are striking. An isotropic phase is formed at 55°C with low water content and high peptide content (p/l 1:15 and 1:7) and also at high water content ($>40\%$, w/w) and high peptide content, at both 35 and 55°C. The phase diagram obtained at 35°C in this study very much resembles the phase diagram obtained at 25°C in [6]. The phase behaviour of the Pgs peptide E/DOPC/^2H_2O system at 35°C is not significantly different from the phase behaviour at 25°C (Morein and Strandberg, unpublished data). Therefore we chose to compare Figs. 4A and 6A despite the difference in temperature.

Unfortunately, we have still not been able to produce any conclusive data on the structure of the isotropic phase that is observed here and in the previous study of the phase behaviour of the Pgs peptide E/PC/^2H_2O systems. X-ray diffraction studies of the sample whose ^{31}P NMR spectrum is shown in Fig. 5B exhibited the regular q-spacings of an L_{α} phase (see Fig. 7), while samples that are shown by ^{31}P NMR to contain an isotropic phase did not show any additional diffraction pattern which can be attributed to any well-known structure. Possibly, the isotropic structure is just composed of aggregated peptides as has recently been observed for similar systems containing transmembrane peptides [22].

Considering the previous studies of transmembrane peptides in lipid bilayers, the almost identical phase diagrams obtained here in the different lipid systems were not expected, since the hydrophobic mismatch in the Pgs peptide E/DEruPC/^2H_2O system should be considerably larger than in the system Pgs peptide E/DOPC/^2H_2O. This would suggest the formation of a H_{II} phase in the presently studied system, as observed for the WALP/PC/^2H_2O systems [12]. Thus, other properties of the peptide than the hydrophobic mismatch must affect the phase behaviour also.

An important point in the hypothesis of hydrophobic mismatch is the physico-chemical properties of the end sites of the peptides located at the hydrophobic–hydrophilic interface. There is evidence that the aromatic amino acid residues Trp and Tyr play an important role in the positioning of transmembrane peptides relative to the membrane surface

(see e.g. [23]). Analyses of amino acid sequences of several single-spanning transmembrane proteins reveal that Trp and Tyr are almost exclusively located in the interface between the hydrophobic and hydrophilic regions [24]. Therefore, it is hypothesised that Trp and Tyr act as sites for locating the ends of the transmembrane peptides relative to the interfacial region. In the WALP peptide (which is designed to be similar to gramicidin A) there are two Trp residues at each flank of the transmembrane part of the peptide which indicates strong affinity of the flanks for the interfacial region of the membrane. In Pgs peptide E the sequence Glu–Tyr at position 2–3 near the N-terminus is a reasonable site for the peptide to be located in the interface between the hydrophobic and the hydrophilic region. Glu is charged at pH 7.0 and is therefore likely to be outside the hydrophobic region of the bilayer and Tyr has an affinity for the interface as stated above. Near the C-terminus in Pgs peptide E there is a Trp residue at position 18 and there is a Tyr residue at position 23 which might act as sites to locate the end of the peptide in the interfacial region. If the same sites in Pgs peptide E are located at the hydrophobic–hydrophilic interface in both DOPC and DEruPC bilayers then it is unlikely that similar phase behaviour would be observed since the difference in hydrophobic thickness of the bilayers is 7 Å (calculated from data presented in [13]). These observations suggest that Pgs peptide E can be differently located in different environments. The dynamic nature of the surface regions of the membrane will most probably result in the peptide being dynamically positioned relative to the hydrophobic–hydrophilic interface. This will of course be constrained by thermodynamic properties of the interaction of the amino acid residues with the different region of the bilayers (e.g. charged amino acid residues will not be located in the hydrophobic region of the bilayer). Most of the transmembrane peptides that have been studied so far do not have large parts that are likely to be outside the hydrophobic region of the bilayer. Therefore, the polar/charged amino acid residues close to the termini of those peptides would be very unlikely not to act as sites to be located in the interfacial region. If the positioning of the peptide in the bilayer is not clearly characterised, then the concept of hydrophobic mismatch cannot be easily applied to explain the lipid/peptide phase behaviour.

The C-terminus of Pgs peptide E is highly amphiphilic and a considerable part of it (up to 10 amino acid residues) is likely to be outside the hydrophobic part of the membrane. The orientation of the C-terminal part of the peptide may have a large influence on the lipid aggregates. It has been shown that surface-associated proteins have considerable influence on the cooperative motions in bilayers of phosphatidylglycerol lipids [25]. This indicates that conformation and position of the C-terminal part of Pgs peptide E might be important factors in the interaction with the lipid bilayer and hence which of the lipid phases will be formed. The interfacial region can comfortably accommodate an α-helix that is oriented along the bilayer [26]. This means that there are different conformations which are possible for the C-terminus of the Pgs peptide E.

The present study indicates that a transmembrane peptide which originates from a naturally occurring protein seems, not surprisingly, to have quite different properties in its interactions with lipid bilayers than the previously studied model peptides. The amino acid sequence of a peptide originating from a naturally occurring protein is often much more heterogeneous than the model peptides, which often consist of a repeating motif. This will most probably affect the interactions with lipid bilayers. A peptide with both a hydrophobic transmembrane part and an amphiphilic or large hydrophilic part most probably interacts with a lipid bilayer in a more complex way than a peptide with only a transmembrane part. Therefore, the simple model often used to explain the phase behaviour of lipid/peptide systems needs to be modified or extended.

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