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Short-chain diacyl phosphatidylglycerols: which one to choose for the NMR structural determination of a membrane-associated peptide from *Escherichia coli*?

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Abstract. Diacyl phosphatidylglycerols (PG) are the major anionic lipids in the *Escherichia coli* membrane. Short-chain dihexanoyl phosphatidylglycerol (DHPG) was previously utilized for the structural determination, by NMR spectroscopy, of the peptide corresponding to the N-terminal membrane anchor of the glucose-specific enzyme IIA (IIA^{Glc}) from *E. coli.* This study explores the possible use of lipid micelles of dioctanoyl phosphatidylglycerol (DOPG) and didecanoyl phosphatidylglycerol (DDPG) as alternatives to DHPG. At a peptide concentration of 1 mM, the minimum peptide/lipid molar ratios required for the formation of the lipid-binding amphipathic helix are approximately 1 : 40, 1 : 5, and 1 : 5 for DHPG, DOPG, and DDPG, respectively. Based on the lipid titration, the critical micelle concentration (CMC) of DHPG was estimated to be ∼50 mM. The ^{1}H spectral linewidths of the peptide bound to a variety of lipid micelles decrease in the following order: DDPG > DOPG > DHPG. The helical regions of the peptide in different anionic lipids were elucidated based on chemical shift indexes (CSI). Residues Leu2-Leu9, Leu2-Val10, and Leu2-Val10 were found to be helical in DHPG, DOPG, and DDPG, respectively, indicating that the lipid chain length had only a subtle effect on the amphipathic helix of the peptide. In light of the minimum peptide/lipid ratio and the spectral linewidth, and the CSI-derived peptide structure, DOPG is proposed as a good compromise for structural studies of this membrane-associated peptide by solution NMR spectroscopy.

Keywords: Micelles, amphipathic helix, lipid chain length, CMC, NMR, *Escherichia coli*

1. Introduction

Membrane proteins account for ∼30% of the proteins coded by the genome, but the number of such structures deposited in the Protein Data Bank is sparse. This paucity of data provides a good opportunity for structural studies by NMR and other biophysical techniques. In the case of solution NMR, single-chain detergent micelles have been widely used. Recently, much attention has been paid to short-chain diacyl phospholipids, which also form micelles [1–4]. In a previous study, we determined the three-dimensional structure of the N-terminal amphitropic domain of enzyme IIA^{Glc}, a phosphocarrier in the

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Escherichia coli glucose transport pathway, in complex with anionic dihexanoyl phosphatidylglycerol (DHPG) [4]. This protein domain is essential for phosphoryl transfer from IIA^{Glc} to the membrane-bound glucose transporter $IICB^{Glc}$ from *E. coli* since deletion of seven residues from the N-terminal domain of IIA^{Glc} leads to loss of 98% of the phosphotransfer activity [5]. Our working model is that this portion of IIA^{Glc} serves as a membrane anchor that stabilizes the entire IIA^{Glc}–$IICB^{Glc}$ complex for efficient phosphoryl transfer in the *E. coli* glucose pathway [6]. Since a membrane-targeting sequence similar to the N-terminal domain of IIA^{Glc} is conserved in eubacteria, archaea, and plants [7], IIA^{Glc} may serve as a useful model for understanding protein amphitropism [4]. Diacyl phosphatidylglycerols (PG) are the major anionic lipids in the *E. coli* membrane and the use of DHPG for structural studies of the *E. coli* membrane proteins may be a good approximation. However, the acyl chains of DHPG each contain only six carbons while the dominant acyl chains of PG in *E. coli* each contain 16 carbons. Naturally, one would ask to what extent the chain length of lipid influences the conformation of the peptide corresponding to the N-terminal domain of IIA^{Glc}. To provide additional insight into this model system, we performed an NMR study of the peptide in three different PG, namely DHPG, dioctanoyl phosphatidylglycerol (DOPG), and didecanoyl phosphatidylglycerol (DDPG). Further extension of PG to the 12-carbon chain or higher is not practical since they form lipid bilayers [8], which are not suitable for solution NMR studies. Our results indicate that the effect of lipid chain length on the conformation of the helical region of the peptide is relatively subtle in the range of lipids we investigated. DOPG appears to be a good compromise for NMR studies of this peptide.

2. Materials and methods

A peptide (99% pure) corresponding to 15 residues of the N-terminal region of IIA^{Glc} from *E. coli* was synthesized by solid-phase methods and purified by reverse-phase HPLC (Peptide Technologies, MD). DHPG, DOPG, and DDPG (>98%) were purchased from Avanti Polar Lipids (Alabaster, AL). Chloroform was removed from the three PG under a stream of nitrogen gas and under vacuum overnight. All lipids were nondeuterated and used without further purification.

2.1. Nuclear magnetic resonance spectroscopy

In all the NMR samples, ∼1.0 mg of peptide (∼1 mM) was solubilized in 0.6 ml of aqueous solution containing 90% H_2O and 10% D_2O at pH 5.4. The minimum peptide/lipid molar ratio in each case was determined by lipid titration into a peptide solution until no significant change in the NMR spectra between two consecutive titrations was observed [4]. NOESY spectra [9] were acquired at a mixing time of 100 ms for each peptide/lipid complex on a Varian INOVA 600 MHz NMR spectrometer using States-TPPI [10]. Typically, spectra were collected with 400 increments (64 scans each) in t1 and 4K data points in t2 time domains using a spectral width of 8510.6 Hz in both dimensions with the ^1H carrier on the water resonance. The data in the t1 dimension was doubled by linear prediction prior to Fourier transformation [11]. The water signal was suppressed by low power (50 Hz) presaturation during both the relaxation delay and the mixing period in NOESY experiments. NMR data were processed as described [4] using NMRPipe [12] to yield a data matrix of 4K × 2K. Because 2,2-dimethyl-silapentane-5-sulfonate sodium salt (DSS) was found to interact with this peptide, DSS was not added to any NMR samples as an internal chemical shift standard [4]. Instead, the peptide signals were referenced to the water signal [4,13], which in turn was referenced to internal DSS at 0.00 ppm [14].

3. Results and discussion

3.1. Titration of phospholipids to the peptide

The peptide sequence is GlyLeuPheAspLysLeuLysSerLeuValSerAspAspLysLys. Figure 1A shows one-dimensional (1D) NMR spectra of the peptide in DHPG, DOPG, and DDPG, all at a peptide/lipid molar ratio of 1 : 1. Although the peptide spectrum in DHPG resembles the spectrum in water [4], the spectra in DOPG and DDPG are clearly different. Both line broadening and shifting of the peptide signals are indicative of peptide/lipid interactions [4]. The aromatic signals of Phe3 moved upfield with an increase in lipid chain length at the 1 : 1 ratio. While the peak pattern of Phe3 in DHPG is similar to that in water, the aromatic signals in DDPG already resemble those in the lipid-saturated state (Fig. 1B). The amide signals of both Leu2 and Phe3 migrated downfield with an increase in lipid chain length (Fig. 1A) and the trend is maintained at saturation ratios (Fig. 1B). Similar spectral changes were previously observed with an increase in the concentration of DHPG before lipid saturation [4], suggesting that the positions of the amide protons of Leu2 and Phe3 are related to both lipid chain length and concentration. The C-terminal residues Asp12, Asp13, Lys14, and Lys15 displayed minimal lipid chain length-dependent shifts at the peptide/lipid ratio of 1 : 1 (Fig. 1A), indicating that these residues do not interact with the lipid. At the saturated peptide/lipid ratios (Fig. 1B), however, these amide signals did change slightly with an increase in lipid chain length, causing Asp12-Lys14 to move upfield and Lys15 downfield, probably due to their electrostatic interactions with the negatively charged micelle surface. Indeed, all residues that showed significant shifts are charged (such as Asp4 and Lys7) or close to the charged residues (such as Ser11) (Fig. 1B). Also, the peak linewidths of the peptide became broader with an increase in the lipid chain length. In DDPG, the amide signal of Asp4 became so broad that it is not readily identifiable on the 1D spectrum. As a consequence of these changes, the signal dispersion for the 7.5–8.5 ppm amide region of the peptide in DOPG or DDPG became poorer compared to the same region in DHPG (Fig. 1B). In addition, the lipid signals showed slight shifts and line broadening in all cases compared to those in water, further suggesting the association of these lipids with the peptide.

For both DOPG and DDPG, the 1D spectra changed little as the ratio was increased from 1 : 5 to 1 : 20 (not shown). Hence, the minimum peptide/DOPG or peptide/DDPG ratio for helix formation is approximately 1 : 5. The peptide signals in DHPG, however, kept changing as the peptide/lipid ratio was increased to 1 : 40 (Table 1). The minimum ratio in DHPG is therefore higher than the minimum ratio of 1 : 10 reported previously [4], which may be attributed to the difference in peptide concentration (5 mM vs. 1 mM in this study). In both cases, however, the concentrations of DHPG at the minimum peptide/lipid ratios are similar (50 mM vs. 40 mM in this study). The minimum ratios found for these lipids are consistent with the order of their critical micelle concentrations (CMC) (Table 1) [8]. We were not able to find the CMC of DHPG in the literature. Based on the lipid titration experiments, we estimate that the CMC of DHPG is $\sim$50 mM (see Table 1), which is about three-fold higher than the CMC of dihexanoyl phosphatidylcholine (DHPC, 15 mM) [8]. Since the minimum peptide/lipid ratio depends on the peptide concentration, in the absence of commercially available deuterated DHPG or isotope-labeled peptides, a useful strategy is to employ higher peptide concentrations, say 5 mM as we did previously [4]. This offers two advantages. First, the signal-to-noise of the peptide signals is improved at a higher peptide concentration. Second, the interference from the strong protonated lipid signals can be reduced by using a lower percentage of lipid.

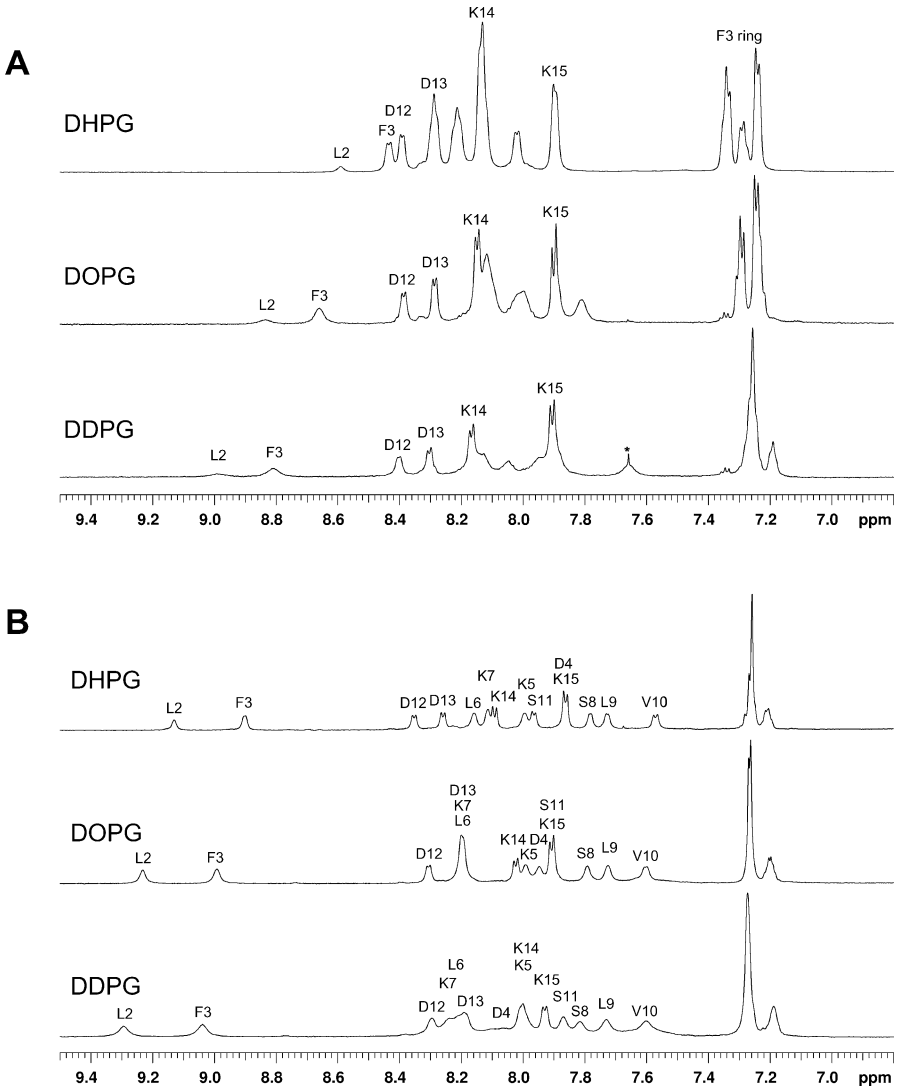

Fig. 1. One-dimensional NMR spectra of the N-terminal membrane anchor of enzyme IIA^{Glc} of *E. coli* (1 mM) in the three different anionic phospholipids. The peptide/lipid molar ratios are 1 : 1 in (A) and 1 : 10 in (B) except for DHPG in (B), where the peptide/DHPG ratio is 1 : 60. Data were collected at pH 5.4 and 25°C. In (A) of DDPG, the sharp signal from residual chloroform signal (on top of the broad amide signal) was labeled with a star. The peptide amide signals are labeled using the single-letter amino acid code.

3.2. NMR signal assignments and secondary structures of the peptide in different PG

On the basis of the lipid titration, a peptide/lipid ratio of 1 : 10 was chosen for both DOPG- and DDPG-containing samples used to collect 2D NMR data. The signal assignments in DOPG (A) or DDPG (B) (Fig. 2) were achieved by using the established method [15] and by comparing them with the known assignments of the peptide in DHPG [4]. The resulting NOE patterns of the peptide in both DOPG and DDPG are almost identical (Figs 2 and 3). Medium H_i^{α}–$H^N{}_{i+1}$, H_i^{α}–$H^N{}_{i+3}$, weak H_i^{α}–$H^N{}_{i+2}$, and H_i^{α}–$H^N{}_{i+4}$ NOE cross peaks were observed for residues Leu2-Val10 (Fig. 3), indicative of helical con-

Table 1

A summary of the NMR data for the peptide from the N-terminal region of the *E. coli* enzyme IIA^{Glc} in short-chain diacyl phosphatidylglycerols

Lipid	Minimum peptide/lipid molar ratio[a]	CMC (mM)[b]	Peptide linewidth (Hz)[c]	Total molecular weight[d]	Peptide helical region[e]
DHPG	1 : 40	∼50	10.37	7268	Leu2-Leu9
DOPG	1 : 5	1.21	12.34	13146	Leu2-Val10
DDPG	1 : 5	0.42	17.20	21299	Leu2-Val10

[a]Determined by lipid titration into a 1 mM peptide solution until little change is observed in the peptide spectra (not shown).

[b]The critical micelle concentration (CMC) of dihexanoyl phosphatidylglycerol (DHPG) was estimated based on lipid titrations. The peptide/lipid minimal ratios were found to be 1 : 10 and 1 : 40 at peptide concentrations of 1 mM (current study) and 5 mM [4]. Since little spectral change in the peptide was observed above these ratios, indicating that the peptide is in the fully bound state. The upper concentration was taken here as the CMC of DHPG because the peptide may have an effect. The CMCs of dioctanoyl phosphatidylglycerol (DOPG) and didecanoyl phosphatidylglycerol (DDPG) are from [8].

[c]The average value of the linewidths (all within ±0.08 Hz) of the two well resolved amide protons of Leu2 and Phe3 of the peptide bound to the PG as measured on 1D NMR spectra (Fig. 1B) using the VNMR software. Similar results were obtained for DOPG and DDPG at peptide/lipid ratios of 1 : 5, 1 : 10, and 1 : 20 and for DHPG at 1 : 40 and 1 : 60.

[d]The total molecular weight for each peptide/lipid complex was calculated on the assumption that one peptide binds to one spherical micelle. Thus, DHPG, DOPG, and DDPG contain 12, 22, and 34 lipid molecules per micelle, respectively. The molecular weights of DHPG, DOPG, and DDPG are 464.4, 520.5, and 576.6, respectively. The total molecular weight of the complex equals the sum of the molecular weights of all lipid molecules in the micelle plus the peptide. The peptide molecular weight from mass spectroscopy is 1695. For other relevant parameters and equations used in the calculations, see [8].

[e]Based on the chemical shift indexes (CSI) [1].

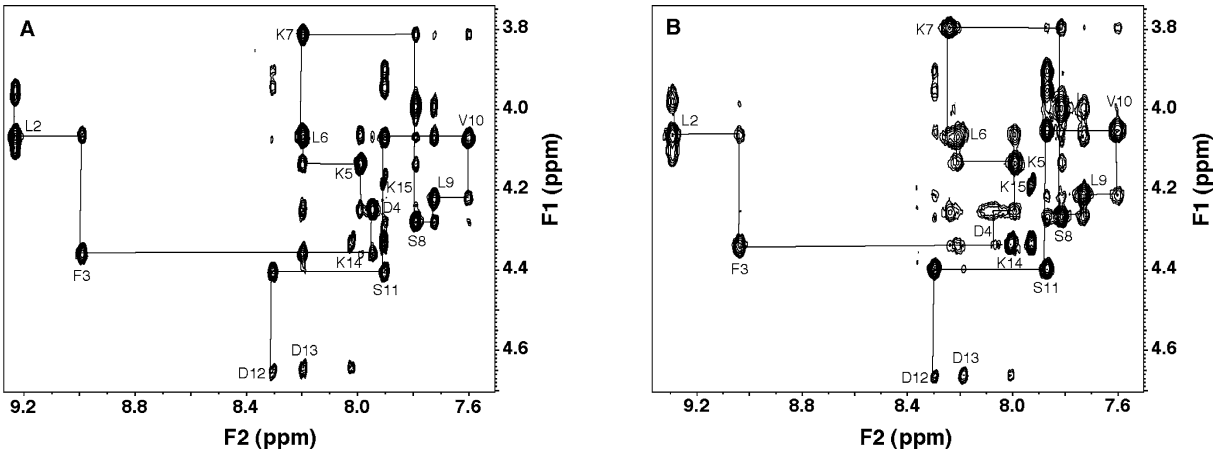

Fig. 2. The fingerprint regions of the NOESY spectra of the N-terminal domain of *E. coli* enzyme IIA^{Glc} in DOPG (A) and DDPG (B), at a peptide/lipid molar ratio of 1 : 10, pH 5.4 and 25°C. The constructs depict the sequential assignments of the peptide signals.

formation [15]. The $d_{N\alpha}/d_{\alpha N}$ NOE ratios for residues Phe3-Val10 are greater than 1, indicating helical conformation (see Fig. 3) [16]. The ratio for residue Leu2 was not calculated due to the signal overlap.

Because chemical shifts are sensitive to local structural change [15,17], we also calculated the chemical shift indexes (CSI) [17] for the α-protons of the peptide in the three PG. CSI are a simplified version of secondary shifts, which are the chemical shift differences between the measured values and the shifts from random-coiled peptides [15]. In the α-proton case, secondary shifts greater than 0.1 are represented as "+1", whereas secondary shifts less than −0.1 are assigned as "−1". All residues with

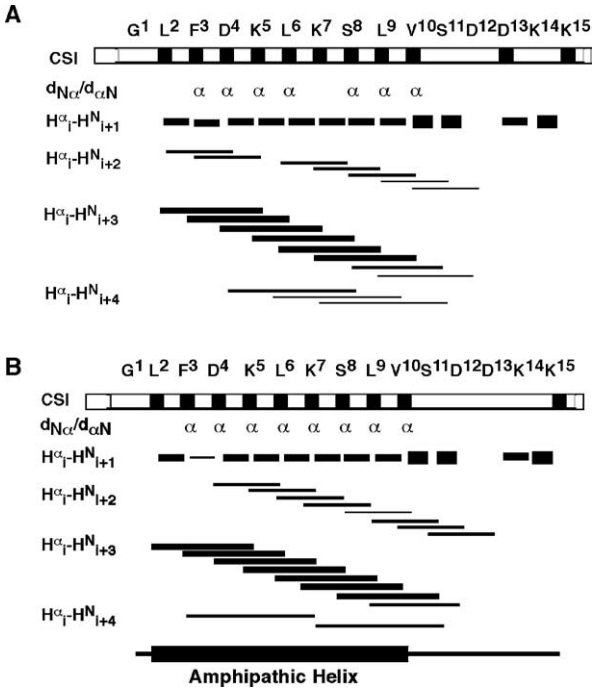

Fig. 3. The secondary structure of the N-terminal membrane anchor ($\sim$1 mM peptide) of enzyme IIA^{Glc} in DOPG (A) and DDPG (B) as indicated by NOE [15], $d_{N\alpha}/d_{\alpha N}$ ratios [16], and CSI [17], at a peptide/lipid ratio of 1 : 10, pH 5.4, and 25°C. In the NOE case, the thickness of the lines is proportional to the volume of NOE cross peaks. For the ratios of $d_{N\alpha}/d_{\alpha N}$, helical structure is indicated by an α for the residue if the ratio is greater than 1. The symbol $d_{N\alpha}$ represents the intra-residue NOE between the amide proton and α-proton of the same residue i while $d_{\alpha N}$ represents the inter-residue NOE between the amide proton of residue i and the α-proton of its preceding residue $(i-1)$ [16]. The solid columns for CSI represent "-1"s, indicating helical conformation [17]. The amphipathic helix is depicted as a black horizontal bar at the bottom.

secondary shifts between -0.1 and 0.1 are assigned "0", indicating random coils. A cluster of "-1"s (upfield shifts) in the plot of these values against residue number is an indication of an α-helix [17]. According to this criterion, residues Leu2-Val10 are helical in both DOPG and DDPG (Fig. 3). In our previous study, residues Leu2-Leu9 were identified as the helical region based on secondary shifts [4]. This is consistent with the presence of signal splitting in the amide proton of Val10 in DHPG (Fig. 1B), indicating flexibility. Nevertheless, the chemical shift change of the α-proton of Val10 in the three lipids is very small (4.14, 4.07, and 4.05 ppm in DHPG, DOPG, and DDPG, respectively). Indeed, structural calculations of the peptide in DHPG indicate that the predominant helical region covers residues Leu2-Val10 [4]. Based on all the structural evidence above, we conclude that PG with lipid acyl chain length in the range of six to ten carbons have only a marginal effect, if any, on the structure of this peptide (Table 1).

3.3. Comparison of the ^1H NMR data of the peptide bound to lipid micelles and the ^{31}P NMR spectra of lipids

The formation of the peptide/DHPG complex was directly supported by intermolecular NOE data [4]. Similar intermolecular NOE cross peaks were also observed between the peptide and DOPG, and between the peptide and DDPG (not shown). As a result of complex formation, the signals of both the peptide and lipid became broader, although the line broadening is greater in the peptide than in the lipid

signals. In addition, the line broadening increased with an increase in the chain length of PG. This is because an increase in lipid chain length resulted in an increase in micelle size, leading to slower tumbling of the complex. For example, the average linewidths of the well-resolved backbone amide signals of Leu2 and Phe3 of the peptide bound to DHPG, DOPG, and DDPG are 10.4, 12.3, and 17.2 Hz, respectively (Table 1). The linewidth increase of the peptide from DHPG to DDPG is also evident from the aromatic side-chain signals of Phe3 in the 1D NMR spectra (Fig. 1B) as well as in the 2D NMR spectra (Fig. 2). While the 2D NOESY quality of the peptide in DOPG (Fig. 2A) is comparable to that in DHPG [4], the line broadening is more evident in the spectrum of the peptide in DDPG (Fig. 2B).

It is interesting to note that a similar trend was observed in a previous ^{31}P NMR study of a series of the PG lipids alone by Kleinschmidt and Tamm [8]. They found that the ^{31}P NMR spectra were sharp and symmetrical in both DHPG and DOPG, typical of isotropic motions. In DDPG, however, the ^{31}P NMR spectrum was not fully symmetrical, indicating that chemical shift anisotropy exists. Finally, the ^{31}P NMR spectrum of the PG with 12-carbon acyl chains appeared as a powder pattern, which indicates that chemical shift anisotropy dominates. Thus, with an increase in lipid chain length, the spectral linewidth change observed in this study by ^{1}H NMR on the *peptide* in complex with lipids and that observed by ^{31}P NMR on the *lipid* alone [8] are correlated. This correlation suggests that lipid micelles dominate the tumbling rate of the peptide/lipid complexes. This is supported by our calculations in Table 1 that assume one peptide bound to one spherical micelle. The peptide molecular weight accounts for 23%, 13%, and 8% of the total molecular weight of the peptide in complex with DHPG, DOPG, and DDPG micelles, respectively. The previous ^{31}P NMR data [8] indicated that a further extension of the PG acyl chain to 12 carbons or more is not practical for solution NMR.

4. Conclusion

A current trend in choosing lipids for structural determinations of membrane proteins or peptides by solution NMR is to use short-chain lipids such as zwitterionic DHPC or anionic DHPG in place of the traditional single-chain detergents such as dodecylphosphocholine (DPC) or sodium dodecylsulfate (SDS). These short-chain lipids are very attractive since not only do they allow high-resolution NMR studies but also tend to preserve the activity of membrane proteins well [18]. In this study, we compared the NMR data of the N-terminal amphitropic domain of IIA^{Glc} bound to the PG micelles of varying chain lengths. We found that the chain length of the PG had only a marginal effect on the amphipathic helix responsible for membrane binding of the N-terminal amphitropic domain of IIA^{Glc} (Table 1).

With an increase of lipid chain length, a parallel linewidth increase observed in both the ^{31}P NMR signals of *lipid* micelles [8] and the ^{1}H NMR signals of the *peptide* bound to lipid micelles suggests that it is the PG micelle that determines the tumbling rate of the peptide/lipid complex. It also suggests that the previous ^{31}P NMR work on lipids [8] may be a useful guide for choosing relevant lipids for structural studies of membrane-associated peptides. A specific lipid head group can be chosen depending on the major lipids in a specific membrane. For the *E. coli* membrane proteins, short-chain phosphatidylglycerols can be chosen as demonstrated by our previous [4] and current studies because PG are the major anionic lipids of this bacterium. For the N-terminal membrane anchor of the *E. coli* enzyme IIA^{Glc}, DOPG appears to be a good compromise for structural studies for three reasons. First, it gives good NMR spectra with relatively narrow spectral linewidths comparable to that in DHPG. Second, the helical region of the peptide in DOPG is identical to that in DDPG on the basis of CSI [17]. Thus, DOPG is safer in case the chain length of the lipid has an effect. Third, it stabilizes the helical structure of the

peptide at a much lower lipid concentration than DHPG. A low lipid concentration allows higher-quality NMR spectra of unlabeled peptides to be collected with less interference from strong protonated lipid signals. In addition, a lower lipid concentration is more economical, especially when deuterated lipids are utilized.

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References

[1] F.M. Marassi, C. Ma, H. Graskowski, S.K. Straus, K. Strebel, M. Oblatt-Montal, M. Montal and S.J. Opella, *Proc. Natl. Acad. Sci. USA* **96** (1999), 14336–14341.
[2] C. Fernandez, C. Hilty, G. Wider and K. Wüthrich, *Proc. Natl. Acad. Sci. USA* **99** (2002), 13533–13537.
[3] J.J. Chou, J.D. Kaufman, S.J. Stahl, P.T. Wingfield and A. Bax, *J. Am. Chem. Soc.* **124** (2002), 2450–2451.
[4] G. Wang, P.A. Keifer and A. Peterkofsky, *Protein Sci*. **12** (2003), 1087–1096.
[5] N.D. Meadow, P. Coyle, A. Komoryia, C.B. Anfinsen and S. Roseman, *J. Biol. Chem*. **261** (1986), 13504–13509.
[6] G. Wang, A. Peterkofsky and G.M. Clore, *J. Biol. Chem*. **275** (2000), 39811–39814.
[7] T.H. Szeto, S.L. Rowland, L.I. Rothfield and G.F. King, *Proc. Natl. Acad. Sci. USA* **99** (2002), 15693–15698.
[8] J.H. Kleinschmidt and L.K. Tamm, *Biophys. J.* **83** (2002), 994–1003.
[9] J. Jeener, B.H. Meier, P. Bachmann and R.R. Ernst, *J. Chem. Phys.* **71** (1979), 4546–4553.
[10] D. Marion, M. Ikura, R. Tschudin and A. Bax, *J. Magn. Reson.* **85** (1989), 393–399.
[11] G. Zhu and A. Bax, *J. Magn. Reson.* **100** (1992), 202–207.
[12] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer and A. Bax, *J. Biomol. NMR* **6** (1995), 277–293.
[13] A.J. Hartel, P.P. Lankhorst and C. Altona, *Eur. J. Biochem.* **129** (1982), 343–357.
[14] J.L. Markley, A. Bax, Y. Arata, C.W. Hilbers, R. Kaptein, B.D. Sykes, P.E. Wright and K. Wüthrich, *J. Biomol. NMR* **12** (1998), 1–23.
[15] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, NY, 1986.
[16] S.M. Gagne, S. Tsuda, M.X. Li, M. Chandra, L.B. Smillie and B.D. Sykes, *Protein Sci.* **3** (1994), 1961–1974.
[17] D.S. Wishart, B.D. Sykes and F.M. Richards, *Biochemistry* **31** (1992), 1647–1651.
[18] J.A. Killian, *Biochim. Biophys. Acta* **1376** (1998), 401–416.