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Effects of detergent alkyl chain length and chemical structure on the properties of a micelle-bound bacterial membrane targeting peptide

Paul A. Keifer,[a] Alan Peterkofsky,[b] and Guangshun Wang[a,*]

[a] *Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, USA*
[b] *Laboratory of Cell Biology, Building 50, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA*

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Abstract

The effects of phospholipid or detergent chain length on the structure and translational diffusion coefficient of the membrane-targeting peptide corresponding to the N-terminal amphipathic sequence of *Escherichia coli* enzyme IIA^{Glc} were investigated by nuclear magnetic resonance (NMR) spectroscopy. Three anionic phospholipids (dihexanoyl phosphatidylglycerol, dioctanoyl phosphatidylglycerol, and didecanoyl phosphatidylglycerol) and four lipid-mimicking anionic detergents (sodium hexanesulfonate, 2,2-dimethylsilapentane-5-sulfonate, sodium nonanesulfonate, and sodium dodecylsulfate) were evaluated. In all cases, the cationic peptide adopts an amphipathic helical structure. While the chain length of the two-chain phospholipids has a negligible effect on the peptide conformation, the effect of chain length of those single-chain detergents on the helix length is more pronounced. The diffusion coefficients of the peptide/micelle complexes were found to correlate with the chain lengths of both the lipid and the detergent groups. Taken together, short-chain anionic phospholipids are proposed to be useful membrane-mimetic models for the structural elucidation of membrane-binding peptides such as cationic antimicrobial peptides. DSS does not form micelles by itself according to the diffusion coefficient data, but it does associate with this cationic peptide. Consequently, both DSS and its analog may be chosen as NMR chemical shift reference compounds depending on the nature of the biomolecules under investigation.

Keywords: Amphipathic helix; Diffusion coefficient; IIA^{Glc}; Lipid chain length; Membrane anchor; Micelle; NMR; Short-chain phospholipids

The development of drug resistance in many infective bacteria has resulted in an urgent need to identify novel antibacterial agents. There is growing interest in the isolation of antimicrobial peptides because drug resistance with these peptides is not yet a major complication in their use [1,2]. Such peptides have been identified in many species such as amphibia, fish, and mammals, including humans. To improve our understanding of these interesting peptides, we have created a computer database [3] to store and analyze the numerous antimicrobial peptides described in the literature. The current version of the antimicrobial peptide database, which can be accessed at http://aps.unmc.edu/AP/main.html, lists 525 peptides, mainly from natural sources. The majority of the peptides (96%) are cationic with 50 residues or less [3]. It is believed that the first step in the antimicrobial action of these cationic peptides is the recognition of the negatively charged membrane surface [4,5].

To better understand the peptide–membrane interaction, structural elucidation of these peptides is essential. However, only 68 antimicrobial peptides (~13 % in the database) have been studied by NMR and very few by X-ray diffraction [3]. Moreover, most of the structures were determined in organic solvents such as trifluoroethanol or small detergent micelles of sodium dodecylsulfate (SDS) or dodecylphosphocholine (DPC)[1] [6–8]. SDS was shown in a previous study to decrease the activity of

* Corresponding author. Fax: +1-402-559-4651.
E-mail address: gwang@unmc.edu (G. Wang).

[1] *Abbreviations used:* DPC, dodecylphosphocholine; DHPG, dihexanoyl phosphatidylglycerol; PG, phosphatidylglycerol; DOPG, dioctanoyl phosphatidylglycerol; DDPG, didecanoyl phosphatidylglycerol; SHS, sodium hexanesulfonate; DSS, 2,2-dimethyl-silapentane-5-sulfonate; SNS, sodium nonanesulfonate.

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a membrane-bound enzyme [9]. In contrast, short-chain phospholipids are very attractive, because they have the same chemical structures as the major lipids in biological membranes [10,11]. Dihexanoyl phosphatidylglycerol (DHPG), a short-chain phospholipid, has the same chemical structure as diacyl phosphatidylglycerols (PGs), the major anionic lipids in bacterial membranes. Recently, we showed that anionic micelles of DHPG produced better NMR spectra for the peptide than did SDS. The three-dimensional structure of the peptide was determined previously in DHPG and in SDS. In both types of micelles, the majority of the structures in the ensemble had helical regions covering residues Phe3–Val10 with the C-terminal tail disordered [12]. A close investigation of NMR structural ensembles revealed that more of the structures in SDS have a slightly longer helix than those in DHPG. The variation in the peptide's C-terminal helical regions in the ensemble of the structures may be attributed to multiple conformations for that region of the peptide in micelles, insufficient amounts of NMR restraints for that region, or both. Interestingly, this observation is consistent with the secondary structures deduced from the secondary shifts of the α protons, which also suggest that the helix is slightly longer in SDS than in DHPG [12]. Therefore, α proton chemical shifts are sensitive probes of the local conformations of proteins [13–15].

Because the predominant phospholipids in bacteria have longer acyl chains, the chain length of PGs used for complex formation may be of interest. This study describes how lipid chain length influences the conformation of the peptide corresponding to the N-terminal membrane-targeting sequence of *Escherichia coli* enzyme IIA^{Glc}. This cationic peptide associates only with anionic lipids [16]. Thus, three PGs with different acyl chain lengths, namely DHPG, dioctanoyl phosphatidylglycerol (DOPG), and didecanoyl phosphatidylglycerol (DDPG), were chosen. Further extension of a PG to 12-carbon chains or higher is not practical since these longer-chain lipids form bilayers rather than micelles [17]. To provide additional insight into these newly developed membrane model systems, we also measured the translational diffusion coefficients of this peptide in different PGs. For comparison, we also included several single-chain anionic detergents: sodium hexanesulfonate (SHS), 2,2-dimethyl-silapentane-5-sulfonate (DSS) sodium salt, sodium nonanesulfonate (SNS), and SDS.

Our investigation was also prompted by the following considerations. If the lipid chain length has little or no effect on the peptide structure, one can use the strategy developed by Opella and colleagues [18] for structural determination of membrane-associating peptides. In their approach, the structure of the peptide is first efficiently elucidated in lipid micelles by solution NMR followed by determination of the peptide orientation in the lipid bilayer by solid state NMR. Our data indicate that the peptide/DHPG complex is smaller than either the peptide/DOPG or the peptide/DDPG complex, while the PG chain length has only a negligible effect on the peptide structure. Thus, the short-chain DHPG is proposed to be useful for the structural determination of bacterial membrane-associating peptides such as antimicrobial peptides by solution NMR.

Materials and methods

Materials

A peptide (99% pure), GLFDKLKSLVSDDKK, corresponding to the N-terminal membrane-targeting sequence of IIA^{Glc} from *E. coli*, was synthesized by solid-phase methods and purified by reverse-phase HPLC (Peptide Technologies, MD). SHS and SNS (98%) were purchased from Sigma–Aldrich (St. Louis, MO). DSS (>99%) was a product of Fluka (Buchs, Switzerland) and SDS (>99%) was purchased from Bio-Rad Laboratories (Hercules, CA). DHPG, DOPG, and DDPG (>98%) were purchased from Avanti Polar Lipids (Alabaster, AL). Chloroform was removed from the three PGs under a stream of nitrogen gas followed by evaporation under vacuum overnight. All lipids or detergents were nondeuterated and used without further purification.

Nuclear magnetic resonance spectroscopy

In all the NMR samples, ~1.0 mg of peptide was solubilized in 0.6 ml of aqueous solution (peptide concentration of ~1 mM) containing 90% H_2O and 10% D_2O at pH 5.4. The only exception was the sample containing DSS, where the peptide concentration was ~5 mM. The pH of each sample was measured directly in the 5-mm NMR tube with a micro-pH electrode (Wilmad-Labglass) and adjusted using microliter aliquots of HCl or NaOH solution. The minimum peptide/detergent molar ratio in each case was determined by detergent or lipid titration into a peptide solution until no significant change between the NMR spectra in two consecutive titrations was observed [12]. All NMR data were collected on a Varian INOVA 600-MHz NMR spectrometer at 25 °C. NOESY spectra [19] were acquired at a mixing time of 100 ms for each peptide/micelle complex using States-TPPI [20]. Typically, spectra were collected with 400 increments (64 scans each) in t1 and 4 K 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 were doubled by linear prediction prior to Fourier transformation. 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 [12] using NMRPipe [21] to yield a data matrix of $4\,K \times 2\,K$. Because DSS interacts with this peptide [12], it was not added to any NMR samples as an internal chemical shift standard. Instead, the peptide signals were referenced to the water signal, which in turn was referenced to internal DSS at 0.00 ppm [22]. The peptide proton signals were assigned using the standard procedure [23] and by comparison with the known assignments in DSS, SDS, and DHPG [12].

Translational diffusion measurements

The diffusion coefficients (D_f) were measured using a longitudinal eddy-current delay (LED) pulse sequence [24,25] modified to improve water suppression by using the WET technique (WET-LED) [26]. D_f was obtained by plotting the intensity ratio of the NMR signals (I/Io) versus the strength of pulsed field gradients using the equation

$$I = I_o \exp[-(\gamma\delta g)^2(\Delta - \delta/3)D_f],$$

where γ is the gyromagnetic ratio of the nucleus (^{1}H), δ is the gradient duration (5 ms), and Δ is the diffusion time (100 ms) between the gradient pulses. The gradient strengths (g) were arrayed by 20 levels from 2 to 45 Gauss/cm using z-axis gradients. Gradient data were processed using VNMR software (Varian Inc.). For each dataset, both integral and peak heights were obtained to fit the equation and the results were similar. Choosing merely the peptide aromatic signals or several peptide regions also gave similar results for the complex.

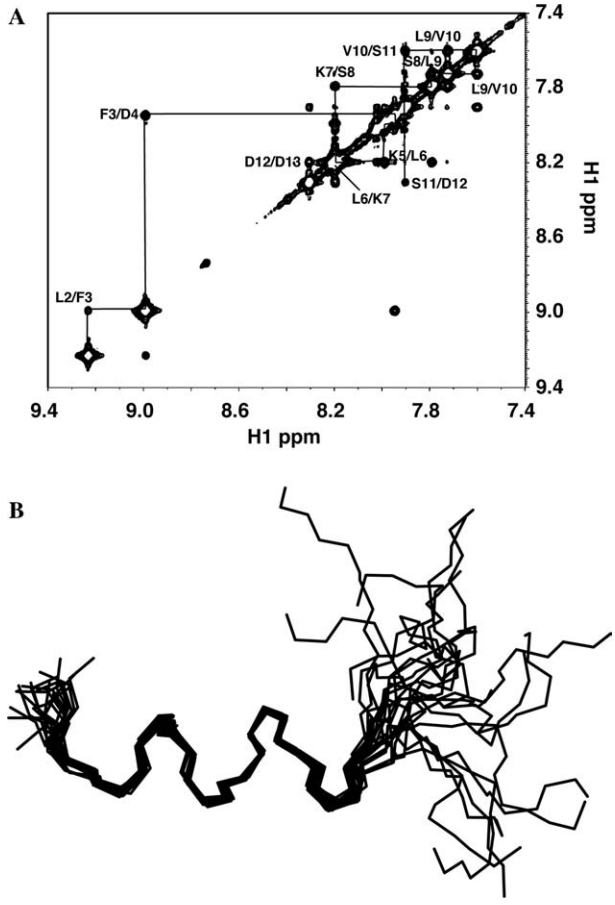

Fig. 1. (A) Amide region of the NOESY spectrum of the peptide corresponding to the N-terminal membrane targeting sequence of *Escherichia coli* IIA^{Glc}, recorded on a 14.0 Tesla Varian INOVA NMR spectrometer in the presence of dioctanoyl phosphatidylglycerol (DOPG) at a peptide:lipid molar ratio of 1:10, pH 5.4, and 25 °C. The NOE walk was constructed with interresidue NOE cross peaks labeled. (B) Three-dimensional structure of the peptide in DOPG. A well-defined helical region, with the backbone atoms superimposed using MOLMOL [37], is found between residues Leu2 and Val10. The C-terminal residues are poorly defined as a result of very few NOE restraints.

Results and discussion

Effect of lipid or detergent chain length on the conformation of the peptide

To further understand the effect of chain length of detergents and short chain anionic lipids on the conformation of the micelle-bound peptide, we have utilized a series of short-chain lipids based on DHPG and another series of detergents using SDS as the template. The three short-chain lipids are DHPG, DOPG, and DDPG, while the three detergents are SHS, SNS, and SDS. The conformations of the peptide in four new micelles (DOPG, DDPG, SHS, and SNS) were investigated as described previously [12]. An example of the signal assignments of the peptide in DOPG, at a peptide/lipid molar ratio of 1:10, pH 5.4, and 25 °C, is presented in Fig. 1A. The strong inter residue backbone amide–amide NOE cross peaks along the peptide chain suggest a helical conformation [23]. This is confirmed by three-dimensional structural calculations using NOE-derived distance restraints and the simulated annealing technique as described [12]. In total, 117 NOE restraints were used. The major helical region covers residues Leu2–Val10 in the ensemble of 20 structures (Fig. 1B). The root mean squared deviation (rmsd) for superimposing backbone atoms ($N-C^{\alpha}-C'$) of this helical region is 0.22 Å. Such a helical region is consistent with that deduced from α proton secondary shifts of the peptide calculated as described previously [12–15] (Fig. 2). As a consequence, we did not pursue additional structural calculations for the peptide in other similar micelles used in this study. Rather, we have calculated the secondary shifts of α protons of the peptide in the three PGs and the three SDS-like detergents. Based on Wishart et al. [14] and [15], a cluster of black bars less than −0.1 in Fig. 2 indicates a helical region. Thus, residues Leu2–Leu9, Leu2–Val10,

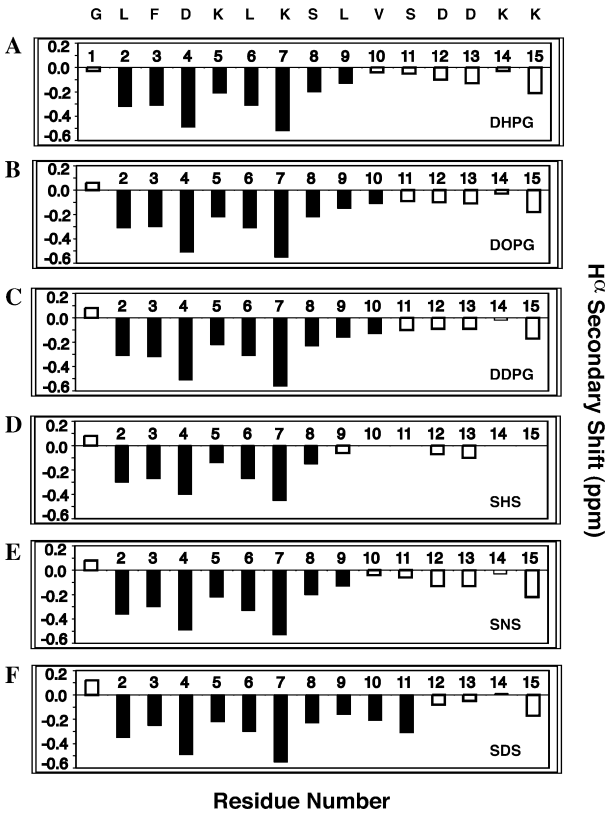

Fig. 2. Secondary chemical shifts of α protons of the peptide in association with (A) DHPG, (B) DOPG, (C) DDPG, (D) SHS, (E) SNS, and (F) SDS, at pH 5.4 and 25 °C. The amino acid sequence of the peptide is depicted at the top. Secondary shifts [14,15] are the chemical shift differences between the measured and the random coil values listed in [23]. The α proton chemical shifts of this peptide were extracted from two-dimensional NOESY spectra at a peptide/detergent molar ratio of 1:10 for all the PGs and 1:500 for SHS, 1:40 for SNS, and 1:20 for SDS. The α proton chemical shift of Lys15 of the peptide in SHS was not found and the secondary shifts for residues Val10, Ser11, and Lys14 of the peptide in SHS (D) are zeros. The peptide concentration was 1 mM, except for the DHPG and SDS cases (5 mM). According to [14,15], a cluster of black bars showing α proton secondary shifts less than −0.1 indicates a helical region.

and Leu2–Val10 of the peptide are helical in DHPG, DOPG, and DDPG, while residues Leu2–Ser8, Leu2–Leu9, and Leu2–Ser11 of the peptide are helical in SHS, SNS, and SDS, respectively. It is evident that the helical region changed little in the PG series (Figs. 2A–C), but the helical region was shortened as the detergent chain length was decreased in the series of single-chain lipid-mimicking detergents (Figs. 2D–F). These results may be related in part to the fact that the acyl chain length of PGs is actually 4 Å longer due to the glycerol backbone effect [27], which does not occur in the case of single-chain detergents. The secondary structures of the peptide are identical in DOPG and DDPG based on the α proton secondary shifts (Fig. 2), suggesting that lipid chain length beyond 10 has no additional effect on the conformation of this peptide (Fig. 1).

Effect of lipid or detergent chain length on the translational diffusion coefficients of the peptide/micelle complexes

To further understand these lipid models, we also measured the diffusion coefficients (D_f) of the micelles by themselves and the peptide in complex with different lipids or detergents listed in Table 1. As anticipated, monomeric detergent molecules ($D_f \sim 5.0 \times 10^{-6}$ cm^2/s) diffuse severalfold faster than their corresponding micelles. Similarly, the peptide by itself ($D_f \sim 2.0 \times 10^{-6}$ cm^2/s) also diffuses severalfold more rapidly than the peptide in the complexes. Titration of SHS into a solution of the peptide showed that the NMR spectra changed little until the SHS concentration approached the critical micelle concentration (570 mM) [28], suggesting that the peptide preferentially binds to a micelle [6,29]. The formation of the peptide/micelle complex is also supported indirectly by peptide signal shifting, line broadening, and directly by intermolecular NOE cross peaks [12]. At lipid concentrations above the minimum peptide/lipid ratio, there is little change in the one-dimensional NMR spectra. Correspondingly, there are minimal changes in the diffusion coefficients of the peptide/micelle complex. For example, the diffusion coefficient of the peptide/DHPG complex is 9.83 (± 0.63)$\times 10^{-7}$ cm^2/s at a peptide/DHPG molar ratio of 1:40, which is nearly identical to the D_f at a molar ratio of 1:60 (Table 1).

To understand the relationship between the diffusion coefficients and the chain length of detergents or lipids, we plotted the D_f against the number of carbons in the acyl or detergent chains. In the absence of the peptide, the diffusion coefficients of the micelles (Fig. 3, squares) decrease with an increase in detergent or lipid chain length, indicative of an increase in the micelle size. Similarly, the diffusion coefficients of the peptide/micelle complexes (Fig. 3, circles) also decrease when the chain length increases. Linear regression analysis showed that the correlation coefficients between D_f and chain length range from 0.92 to 0.99 (see Fig. 3 legend). The two lines describing the D_f–detergent–chain-length correlations with and without the added peptide are not parallel, with the separation between the lines increasing significantly from SDS, SNS, to SHS (Fig. 3A). A possible reason for this may be that the peptide/SHS complex is much larger than the SHS micelle at 1.5 M, which is almost threefold its critical micelle concentration [28]. In contrast, the two lines in Fig. 3B are nearly parallel, indicating that the peptide decreased the diffusion coefficients of the series of PG micelles to a similar extent. These linear correlations suggest that the peptide binds to a series of PG micelles of varying sizes. The shorter the chain length of the PG, the larger the D_f, the smaller the peptide/PG complex, and the narrower the NMR lines of the peptide. Indeed, the average line widths of the well-resolved amide proton signals of Leu2 and Phe3 of the peptide

Table 1
Diffusion coefficient and structure of the N-terminal membrane anchor of *Escherichia coli* enzyme IIA^{Glc} in complex with a variety of lipids or lipid-mimicking detergents at pH 5.4, 25 °C[a]

Detergent[b]	Chain length	$D_f^{detergent}$ ($\times 10^{-7}$ cm^2/s)[c]	$D_f^{complex}$ ($\times 10^{-7}$ cm^2/s)[d]	Helical region
SHS	6	18.9 ± 1.3[b]	9.47 ± 0.29[c]	2–8
SNS	9	9.85 ± 0.51	8.51 ± 0.12	2–9
SDS	12	6.70 ± 0.04	6.07 ± 0.24	2–11
DSS	7	44.8 ± 1.3	8.50 ± 0.25	2–9
DHPG	2×6	12.2 ± 0.35	9.41 ± 0.25	2–9
DOPG	2×8	8.68 ± 0.08	7.02 ± 0.28	2–10
DDPG	2×10	6.55 ± 0.56	5.07 ± 0.35	2–10

[a] All abbreviations are defined in the text.

[b] Detergent abbreviations (molecular weights) and concentrations: SHS (188.2) 1500 mM; SNS (230.3) 500 mM; SDS (288.4) 100 mM; DSS (218.3) 300 mM; DHPG (464.4) 200 mM; DOPG (520.5) 20 mM; DDPG (576.6) 20 mM. While the diffusion coefficients of DSS changed little from 1 to 300 mM (see text), the $D_f^{detergent}$ values for SHS did change with concentration (4.92×10^{-6} cm^2/s at 500 mM, 1.89×10^{-6} cm^2/s at 1.5 M, and 4.68×10^{-7} cm^2/s at 3.0 M). Hence, DSS has limited solubility in water and does not form micelles at a saturated concentration at pH 5.4 and 25 °C, whereas SHS does form micelles at higher concentrations above the critical micelle concentration.

[c] Errors for the D_f were calculated from the difference between the maximum and the minimum values divided by two.

[d] Peptide/detergent molar ratio for each complex: SHS 1:500; SNS 1:40; SDS 1:100; DSS 1:40; DHPG 1:60; DOPG 1:20; DDPG 1:20. All complexes contain 1 mM peptide except for the DSS complex, which is 5 mM. The D_f for 5 mM peptide in complex with SDS at ratios of 1:10 and 1:40 are 6.16×10 and 5.90×10^{-7} cm^2/s, respectively. The diffusion coefficients of the peptide are 1.97×10^{-6} cm^2/s at a peptide/SHS molar ratio of 1:20 (20 mM SHS) and 1.25×10^{-6} cm^2/s at a peptide/SHS molar ratio of 1:320 (320 mM SHS).

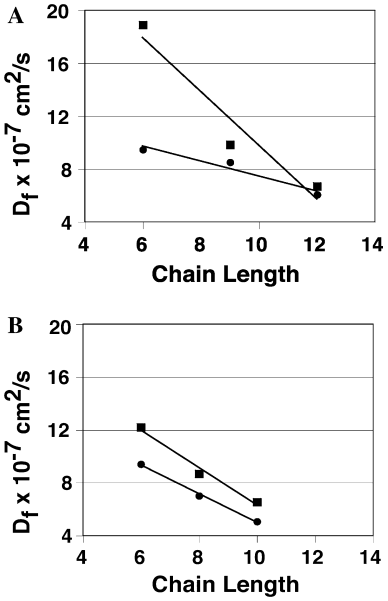

Fig. 3. Correlations between the diffusion coefficients of either the micelles or the peptide/micelle complex and the chain length of either the single-chain detergents or the two-chain lipids. The slopes (correlation coefficients R^2) from linear regression analysis for (A) detergent micelles (squares) and detergent/peptide complexes (circles) and (B) lipid micelles (squares) and lipid/peptide complexes (circles) as a function of chain length are −2.03 (0.927), −0.567 (0.941), −1.412 (0.980), and −1.09 (0.997), respectively. The chain length is represented by the number of carbon atoms in the detergent or lipid acyl chains (Table 1).

bound to DHPG, DOPG, and DDPG micelles measured from one-dimensional NMR spectra are 10.4, 12.3, and 17.2 Hz, respectively.

Except for DSS (see below) and SHS, the diffusion coefficients for the peptide/detergent complexes are only 10–23% less than those of the corresponding micelles (Table 1), suggesting that it is the detergent micelle that determines the diffusion rate of the entire complex. Similar results were obtained for several membrane-surface-binding peptides, including melittin in DPC [6], opioid peptides bound to DPC [30], and a fragment of human apolipoprotein C-I in complex with SDS [31]. In addition, two transmembrane peptides were also shown to have little effect on the size of the SDS micelle [32]. It appears to be general that the micelles dominate the diffusion rate of the peptide/micelle complexes irrespective of the type of peptides. These observations make sense because the molecular weight of those peptides (<3.5 kDa) is less than that of either the SDS or the DPC micelle (~20 kDa) [6,29].

Vinogradova et al. [9] measured the diffusion coefficients of a 39-kDa trimeric *E. coli* membrane protein, diacylglycerol kinase, in complex with a variety of detergents. The diffusion coefficients were found to fall within the narrow range of $2.7–4.7 \times 10^{-7}$ cm^2/s and showed no correlation with the micelle size or chain length. It was concluded that the large membrane protein dominates the diffusion rate of the entire protein/detergent complexes. Although this conclusion is different from that presented here, these two findings are complementary because the two studies represent two different scenarios. In our case, the size of the micelles is much larger than that of the peptide, whereas in their case the size of the protein is more or less comparable to that of the micelles. This can be seen from the differences in the magnitude of the diffusion coefficients. Their diffusion coefficients of the protein/micelle complexes are consistently smaller than those we reported in Table 1 for the peptide/micelle complexes, while the diffusion coefficients of the only common SDS micelle without polypeptides in both studies are identical (6.7×10^{-7} cm^2/s).

DSS and the choice of chemical shift reference compounds

DSS, a recommended chemical shift reference compound [22], was observed to interact with the peptide as evidenced by (1) the line broadening of both the DSS and the peptide signals and (2) the unique intermolecular NOE cross peaks between the DSS and the peptide at 0.00 ppm [12]. To further understand the interaction of DSS with this peptide, we have also measured its translational diffusion coefficient in the free and bound states. In the absence of the peptide, the diffusion coefficient of DSS decreased only slightly from $\sim 5.0 \times 10^{-6}$ cm^2/s at 1 mM to 4.5×10^{-6} cm^2/s at 300 mM (close to saturation at 25 °C, pH 5.4), and a similar value was obtained at a saturated DSS concentration, indicating that DSS did not form micelles by itself under the conditions that we measured. The diffusion coefficient of the peptide, however, decreased significantly at a peptide/DSS ratio of 1:40 (200 mM DSS) and is comparable to the diffusion coefficients of the same peptide in the two series of detergents listed in Table 1. A similar diffusion coefficient (8.7×10^{-7} cm^2/s) was obtained for the peptide at a saturated level of DSS. These measurements indicate that the aggregation of DSS has been greatly enhanced in the presence of this cationic peptide, which neutralizes the negative charge of DSS and thus reduces the unfavorable electrostatic repulsions between DSS head groups. It is also possible that two or more peptide molecules may be incorporated into the same peptide/DSS complex.

DSS also interacts with other peptides [33]. Another chemical shift standard, (trimetylsilyl)propionic acid, was shown to interact with a fragment from the Alzheimer's disease-related Aβ peptide [34]. A solution was proposed by changing the anionic head group of DSS to a cationic group [33]. Alternatively, the alkyl chain of DSS can be shortened to reduce its binding to membrane-targeting peptides. Perhaps, the chemical shift reference compound should be chosen on the basis of the molecular system under investigation to prevent a direct interaction between the standard and the compound to be investigated. For acidic molecules such as nucleic acids (DNA and RNA), DSS may be perfect, while the above DSS analogue [33] may be chosen for basic molecules such as membrane-binding cationic peptides.

Conclusions

The cationic peptide corresponding to the N-terminal surface-binding membrane anchor of enzyme IIA^{Glc} is essential for efficient phosphoryl transfer from this enzyme to the glucose transporter in *E. coli* [16,35]. A similar membrane-targeting sequence is conserved in eubacteria, archaea, and chloroplasts [36]. Therefore, the cationic peptide investigated here may serve as a useful model for understanding interactions of those homologous cationic peptides with anionic bacterial membranes. It is also a good model peptide for understanding the correlation between peptide structure, lipid chain length, and complex size (Figs. 2 and 3). We demonstrate for the first time that the size of the peptide/PG complex depends on the PG acyl chain, although the generality of this conclusion need be validated with other peptides. Smaller complexes such as peptide/DHPG are more favorable for NMR studies because they tumble more rapidly in solution, leading to narrower linewidths. Further, the diacyl phosphatidylglycerols of varying chain lengths that we investigated here had only a subtle effect on the structure of this membrane anchor. Similar helical structures for a similar peptide also occur in dioleoyl phosphatidylglycerol and in a mixture of *E. coli* lipids [16]. Taken together, these findings suggest that short-chain phosphatidylglycerols are useful as membrane-mimicking agents for structural studies of membrane-associating peptides such as the hundreds of cationic antimicrobial peptides [3], which selectively interact with the negatively charged membrane surface.

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