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Specific Ion Interaction Dominates over Hydrophobic Matching Effects in Peptide–Lipid Bilayer Interactions: The Case of Short Peptide

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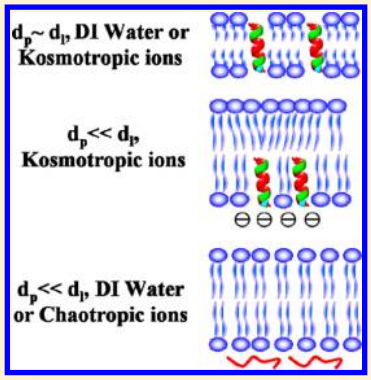
Supporting Information

ABSTRACT: Insertion of short peptides into the cell membrane is energetically unfavorable and challenges the commonly accepted hydrophobic matching principle. Yet there has been evidence that many short peptides can penetrate into the cells to perform the biological functions in salt solution. On the basis of the previous study (*J. Phys. Chem. C* **2013**, *117*, 11095−11103), here we further performed a systematic study on the interaction of mastoparan with various neutral lipid bilayers with different lipid chain lengths in situ to examine the hydrophobic matching principle in different aqueous salt environments using sum frequency generation vibrational spectroscopy. It is found that the hydrophobic matching is the dominant driving force for the association of MP with a lipid bilayer in a pure water environment. However, in a kosmotropic ion environment, the hydration of ions can overcome the hydrophobic mismatching effects, leading to the insertion of MP into lipid bilayers with much longer hydrophobic lengths. When the hydrophobic thickness of the bilayer is much longer than MP's hydrophobic length, MP diffuses on a single monolayer, rather than spanning the bilayer to prevent the exposure of the hydrophilic part of MP to the lipid hydrophobic moiety. Findings from the present study suggest that the interaction between the positively charged choline group of a lipid and kosmotropic ions could be an important step for effective peptide insertion into a cell membrane. Results from our studies will provide an insight into how the short peptides form the ion channel in a thick membrane and offer some ideas for cellular delivery.



INTRODUCTION

It has been more than 100 years since the influence of ions on the protein solubility was found to follow a well-known Hofmeister series: $HPO_4^{2-} > CO_3^{2-} > SO_4^{2-} > S_2O_3^{2-} > H_2PO_4^- > F^- > Cl^- > Br^- > NO_3^- > I^- > ClO_4^- > SCN^-$.[1−6] The mechanism still remains elusive thus far at the molecular level. Moreover, interactions between proteins (peptides) and cell membrane are very important in the biological activities and functions;[7−9] thus, it will be critical to understand how the ions affect such interactions because the ions exist universally in the world and in the human body.[10] To approach this problem, Ninham and his colleagues have performed a theoretical study to investigate the effect of Hofmeister effects on the binding of peptides to model membranes and found that the ionic dispersion potentials are required for the binding energies.[11] However, to the best of our knowledge, there is no systematic experimental research on this issue. Of course, the influence of ions on the interactions between proteins (peptides) and membranes is very complex because the ions can affect the membrane and the peptide molecules at the same time.[12,13] It will be quite difficult to separate these two effects. Therefore, a detailed and systematic study is highly necessary to understand such complex interactions. As a first step of the systematic study, using mastoparan (MP) as a modeling peptide, we recently performed a study on the interactions between a short helical peptide and different charged lipid bilayers of DMPC (neutral), DMPG (negatively charged), and DMEPC (positively charged) in the presence of phosphate ions using sum frequency generation vibrational spectroscopy (SFG-VS).[14] Our study indicated that phosphate ions can surprisingly favor positively charged MP to interact with different kind of charged (negatively, neutrally, positively) lipid bilayer.[14] In general, a α-helical MP is about 2.1 nm long,[15] close to the hydrophobic length (2.3 nm) of DMPC, DMPG and DMEPC bilayers. In addition, MP can form ion channels in membranes at high ionic strength (>0.3 M).[16] Hence, the previous study points out many new questions: for example: How do other ions in the Hofmeister series affect the peptide–membrane interaction? How do the ions affect the interaction when the peptide hydrophobic length is much shorter than the membrane hydrophobic length? Is the phosphate moiety of lipid headgroup essential for the interaction processes?

On the other hand, it has been known that hydrophobic matching in lengths between peptides and lipid bilayers is the major requirement for the insertion of peptides into membranes.[9,17] It is energetically unfavorable to expose the hydrophobic residues to water or hydrophilic amino acids to

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the acyl chains of the lipid hydrophobic core.[9,17] When a hydrophobic matching is not satisfied, either the lipid bilayers or peptides can undergo many adjustments to match each other. For example, the lipid bilayer can change its length by compressing, stretching, and deforming the lipid acyl chains, while the peptide can change its orientation angle in membrane or rotate side chains to fit to the bilayer.[18,19] Normally, if the hydrophobic mismatching difference is too large, the peptide may lie at the membrane interface,[9,17,20] instead of inserting into the membrane.[21] Therefore, to further understand the ionic effects on the protein−membrane interaction, it is necessary to perform a more systematic and detailed study. In this study, we further examined the mastoparan−membrane interaction by varying the lipid chain length and the salt types, as well as considering the lipid without phosphate moiety on the basis of the previous study[14] using SFG-VS. It was found that the influence of kosmotropic anions dominates over the hydrophobic matching effects. Results from our studies will provide an insight into how the short peptides form the ion channels in the thick membrane and offer some ideas for cellular delivery.

2.1. EXPERIMENTAL SECTION

2.1. Materials. MP (sequence: Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2, purity >98%) was purchased from Shanghai Apeptide Co., Ltd. The schematic of the secondary structure of mastoparan with charged units in green, hydrophobic units in red and the neutrally hydrophilic unit in cyan is shown in Figure 1A. The lipids of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-*sn*-glycerol-3- phosphocholine (DMPC), 1,2-dimyristoyl(d54)-*sn*-glycero-3- phosphocholine (d-DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl(d62)-*sn*-glycero- 3-phosphocholine (d-DPPC), 1,2-distearoyl -*sn*-glycero-3-phosphocholine (DSPC), 1,2-dimyristoyl-3- trimethylammonium-propane (chloride salt) (DMTAP), and 1,2-dimyristoyl-3-dimethylammonium-propane (DMDAP) were purchased from Avanti Polar Lipids (Alabaster, AL). Dibasic potassium phosphate (K_2HPO_4), monobasic potassium phosphate (KH_2PO_4), potassium sulfate (K_2SO_4), potassium fluoride (KF), potassium chloride (KCl), potassium bromide (KBr), potassium iodide (KI), and potassium nitrate (KNO_3) were purchased from Aldrich with a purity of >99.8%. All of these salts were baked at around 500 °C for more than 6 h in order to remove the organic impurities.

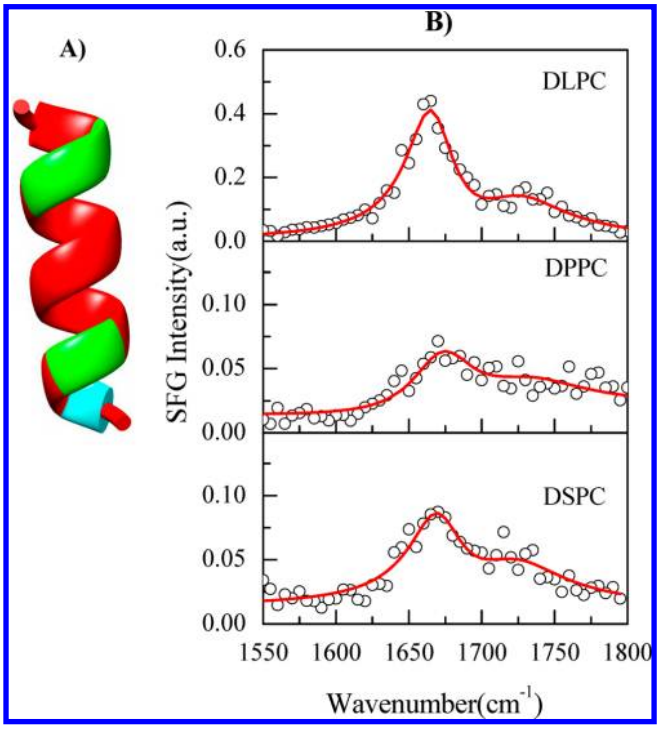

Figure 1. (A) Schematic of secondary structure of mastoparan with charged units in green, hydrophobic units in red and neutrally hydrophilic unit in cyan. (B) Amide-I ssp spectra of MP molecules (with concentration of 30 μg/mL) when interacting with the lipid bilayers of different lipid chain length in pure water environment.

2.2. Sample Preparation and SFG-VS Experiments. We performed the sample preparation and SFG-VS experiments using the standard procedures given in reference 14. In addition, SFG theories and instruments have been introduced previously.[22−32] We will not repeat here to avoid the overlap.

3. RESULTS AND DISCUSSION

3.1. The Case for Lipid Bilayer with Different Lipid Chain Length in Pure Water Environment. The molecular structure of MP in neutrally charged DMPC bilayer in pure water environment has been discussed in our recent study.[14] Similar to the experiments in reference 14, here we investigated the MP molecular structure when interacting with the lipid bilayer with different lipid chain lengths. Figure 1B shows the ssp spectra of MP (with concentration of 30 μg/mL) in different chain length lipid bilayer in pure water environment. A resonance peak was observed at 1665−1675 cm^{-1}, indicating MP adopts a turn structure on the bilayer surface (The fitting parameters of the spectra in Figure 1B were given in Table S1 in Supporting Information [SI]). The SFG spectra intensity of MP in DLPC bilayer is several times higher than the intensity in other lipid bilayers with longer lipid chains. Lipid chain length can modulate hydrophobic mismatch between the protein/peptide and the lipid membrane.[33,34] Table 1 lists the hydrophobic length[35] and transition temperature[36] of the lipid bilayers. The hydrophobic length is taken from the X-ray diffraction data.[35] As indicated by Table 1, the hydrophobic mismatching between MP and DLPC bilayer is smallest, which will benefit the interaction between MP and DLPC bilayer, resulting in a higher SFG intensity in the MP amide I signal. For other lipid bilayers, the hydrophobic mismatching difference is too large to allow MP to incorporate into the membrane. On the other hand, the phase of the lipid bilayer at the room temperature is mainly determined by the lipid chain length: Similar lipids with shorter chains tend to exist in the fluid phase, while with longer chains are likely in the gel phase.[37] Therefore, lipid fluidity may also affect the insertion of mastoparan into lipid bilayer because DLPC has a much higher fluidity compared to the other three lipids. Temperature-

Table 1. Hydrophobic Length of the Lipid Bilayers

Lipid name	Acyl chains	Hydrophobic length(nm)	Transition temperature(°C)[36]
DLPC	C12:0	1.95	−1
DMPC	C14:0	2.3	23
DPPC	C16:0	3.6	41
DSPC	C18:0	4.05	55

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dependent experiments are needed to distinguish the effect caused by the hydrophobic mismatching and the lipid fluidity in the future.

3.2. The Case for Lipid Bilayer with Different Lipid Chain Length in Phosphate Buffer Solution. After investigating the interactions between MP and different chain-length lipid bilayer in pure water, we then added phosphate buffer into the subphase to examine the effect of phosphate ions on the MP interaction with the bilayer with different lipid chain length. Figure 2 shows the SFG spectra of

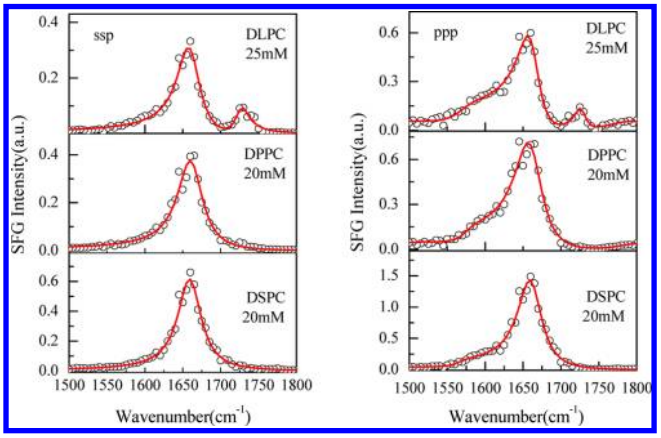

Figure 2. Amide-I SFG spectra of MP molecules (with concentration of 30 μg/mL) when interacting with a lipid bilayer in the presence of phosphate buffer solution with the concentration of 20−25 mM and pH of ∼7.0.

MP molecules (with concentration of 30 μg/mL) at the phosphate buffer solution with the concentration of 20−25 mM. The spectra of MP in DLPC, DPPC and DSPC bilayers at other buffer concentration are given in the SI (Figure S1). Similar to the result in DMPC bilayers,[14] all the spectra are dominated by a strong peak at ∼1655 cm^{-1}. In DLPC bilayers, in addition to the ∼1655 cm^{-1} peak, a weak peak at ∼1720 cm^{-1} originated from the lipid carbonyl group was detected. It is worth noting that the hydrophobic length of DPPC and DSPC bilayer is almost 2 times of MP hydrophobic length. However, even at very low salt concentration, MP can not only interact with DLPC and DMPC bilayers, but also with DPPC and DSPC bilayers. This result suggests that influence of phosphate ions dominates over hydrophobic matching effects in MP-lipid bilayer interactions. Figure 3 shows the results of the fitting ssp intensity change using eq S2 in SI. The buffer concentration dependence of the fitting amplitudes in different lipid chain length follows different trends: a small change in DLPC bilayers, an exponential growth in DMPC bilayers,[14] decrease in DPPC and DSPC bilayers.

To access a precise molecular description of MP's behavior in a membrane, it is necessary to determine the orientation of MP in a lipid bilayer and to know how an α-helical MP molecule spans the phospholipid bilayers. The detailed SFG data analysis methods have been presented in reference 14. A relation between θ for α-helical MP molecules and the measured $\chi^{(2)}_{ppp}/\chi^{(2)}_{ssp}$ ratio is given in the SI (Figure S2). Here the measured $\chi^{(2)}_{ppp}/\chi^{(2)}_{ssp}$ ratios of the ∼1655 cm^{-1} peak all fall in the range of 1.4−1.55, giving an orientation angle θ of 20°−31°, considering a δ-distribution.

After obtaining the orientation information of MP molecules in a membrane, we used an isotopically symmetric d-DPPC

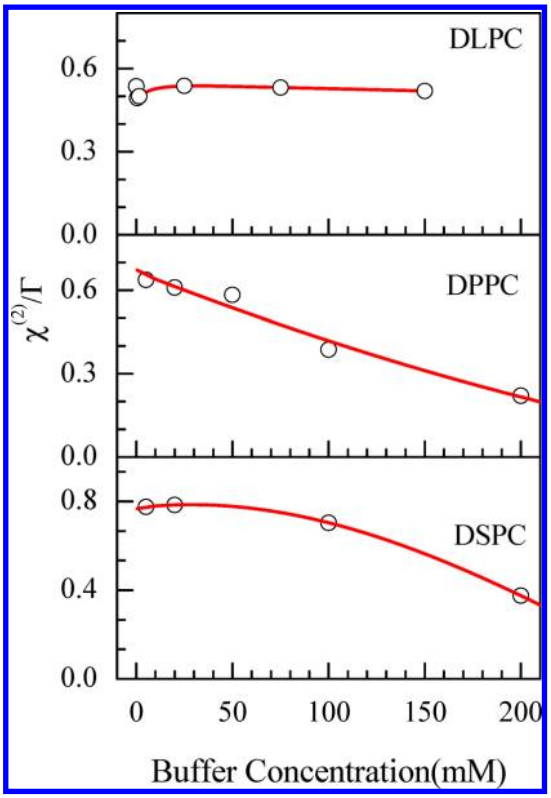

Figure 3. Fitted amplitude of $\chi^{(2)}_{ssp}$ (1655 cm^{-1}) of MP molecules (with concentration of 30 μg/mL) when interacting with different lipid bilayers is plotted as a function of phosphate buffer concentration. The fitting curve (red line) is for guiding the reader's eyes and does not have any physical meaning.

bilayer to differentiate the SFG signal of the MP side chains from those of the lipid bilayer so that we can determine how the α-helical MP molecules affect the structure of lipid bilayers. An increase of SFG signal from the terminal methyl group of the lipid acyl chain will indicate a symmetry breaking of the bilayer caused by MP. Figure 4 presents the ssp spectra of d-DPPC monolayer and the bilayer in the frequency range of 2000−2300 cm^{-1} after MP molecules (with concentration of 30 μg/mL) was injected into the subphase of the lipid bilayer in

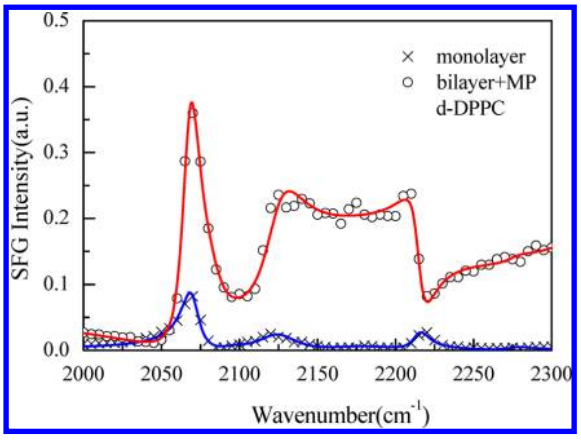

Figure 4. The ssp spectra of d-DPPC monolayer and the bilayer (prepared in 5 mM phosphate buffer solution) following addition of 30 μL MP solution (2 mg/mL in methanol) into the buffer subphase (∼ 2.0 mL) of lipid bilayer.

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the presence of phosphate ions. For the d-DPPC monolayer, the spectra were dominated by the peaks at 2075, 2120, and 2215 cm^{-1}, which are assigned to symmetric stretch, Fermi resonance, and asymmetric stretch of CD_3 group, respectively.[24,38] Weak signals from the CD_2 and CD_3 groups (without MP) have been shown in reference 14, suggesting the structures of both leaflets of the bilayer are very symmetric. After adding MP molecules and reaching equilibrium, the intensity of the ~2075 cm^{-1} peak in the MP-inserted lipid bilayer is 3−4 times of the intensity of the monolayer. For the DPPC and DSPC bilayer, MP's hydrophobic segment is not long enough to contact the inner leaflet of the lipid bilayer. Therefore, this effect is most likely caused by MP molecules penetrating into the outer leaflet of the lipid bilayer and occupying the methyl group position of d-DPPC at the distal leaflet. Thus, the symmetry of the bilayer is broken, leading to the CD_3 signals. In fact, a recent report also supported the peptide diffusing onto a single monolayer, rather than spanning the bilayer when the membrane thickness is much larger than the peptide length.[39] Such adjustments can effectively prevent the exposure of the hydrophilic segments of the peptide to the lipid hydrophobic moiety.

It is well-known that the intensity of the SFG signal is determined by the averaged orientations, Fresnel coefficients, and the surface molecular density (SMD). With considering the influence of averaged orientation and Fresnel coefficient, a change in SMD between the lipid monolayer and the MP-inserted bilayer can be determined by comparing spectral intensities. The orientation angle of the methyl group (θ_{CD_3}) at the tail group of d-DPPC can be deduced according to the relationship between θ_{CD_3} and the ratio of $\chi^{(2)}_{ssp}(CD_3,as)/\chi^{(2)}_{ssp}(CD_3,ss)$ (Figure S3 in SI).[40] Under current experimental geometry, the Fresnel factor at the water interface is about 0.86 times the value at the air surface. Then by comparing the intensities in Figure 4, we can calculate that the SMD of the CD_3 group of the MP-inserted lipid bilayer at the water interface is about 1.8 times the one in the monolayer at the air surface (Table S2 in SI), indicating a local accumulation of lipids after MP interacts with the lipid bilayer. The present study mainly focuses on how the ions affect hydrophobic matching effects. A detailed study on MP-induced local accumulation of lipids will be presented in the next paper.

3.3. The Case for DMPC Lipid Bilayer in Different Salt Solutions. As shown in the preceding section, in phosphate solution, MP can interact not only with DLPC bilayer (with smaller hydrophobic mismatch) but also with DPPC and DSPC bilayers which have a larger hydrophobic mismatch difference of >1.5 nm. This result naturally raises another question as to why MP can interact with a lipid bilayer without satisfying the hydrophobic matching principle in phosphate solution. To address this question, we further investigated the interaction between MP and DMPC bilayers in different ion solutions. Figure 5 shows the ssp SFG spectra of MP molecules (with concentration of 30 μg/mL) in the presence of different salt solutions with the ion strength of ~100 mM and pH of ~7.0. Similar to the results in phosphate solution,[14] the SFG spectra also show the same spectral features in sulfate, fluoride, and chloride solution environments, namely, the spectra are dominated by a strong peak at ~1655 cm^{-1}, and a weak peak at ~1720 cm^{-1}. However, the spectra show a broad peak originated from the membrane-bound water in bromide, iodide, and nitrate solutions. This result suggests that MP can interact

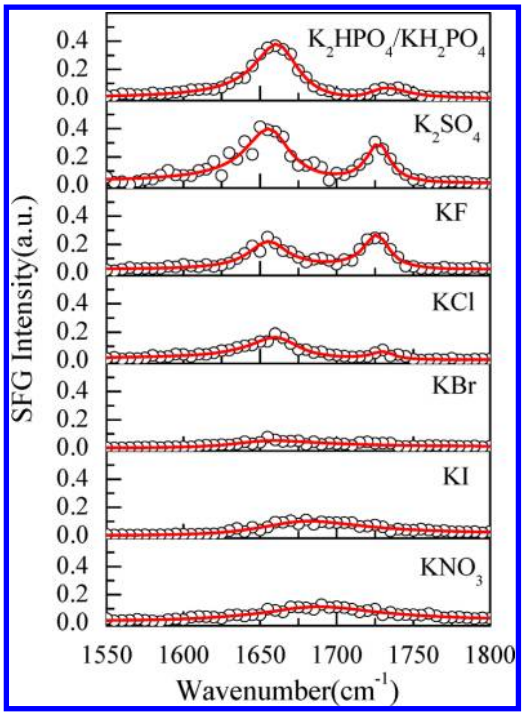

Figure 5. Amide-I ssp SFG spectra of MP molecules (with concentration of 30 μg/mL) when interacting with DMPC lipid bilayer in the presence of different buffer solutions with the ion strength of ~100 mM and pH of ~7.0.

with a lipid bilayer in the presence of phosphate, sulfate, fluoride, and chloride ions but cannot in the presence of bromide, iodide, and nitrate ions. As stated in many Hofmeister studies,[1−6] kosmotropic ions (HPO_4^{2-}, CO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $H_2PO_4^-$, F^-, Cl^-) can prevent protein unfolding by precipitating proteins from solution, whereas chaotropic ions (Br^-, NO_3^-, I^-, ClO_4^-, SCN^-) can increase the protein solubility and accelerate the protein denaturation. Thus, protein stability is often linked to the hydration behavior of ions.[5,6] Furthermore, kosmotropic ions can affect lateral packing of the lipid molecules and thus the bilayer lateral pressure profile by changing the interfacial tension or lessening the osmotic water at the lipid hydration layer.[41] The influences of a specific ion on either the lipid bilayer or the peptide molecules may both contribute to peptide insertion into the lipid bilayer. In order to achieve more in-depth understanding in the nature of the ion effect and its direct relation to the hydrophobic mismatching, further experiments are needed in the future to examine these two effects separately.

Since the ion's hydration has been related to the hydration Gibbs free energy (kJ/mol), no matter how the ions affect the lipid bilayer and peptide molecules,[42] we plotted the fitting amplitudes of the ssp spectra in Figure 5 as a function of hydration Gibbs free energy (Figure 6). A linear relationship deduced from Figure 6 indicated that an ion's hydration is the dominant driving force for MP-DMPC bilayer interaction.

On the other hand, phosphate groups in the lipid membrane are hydration centers;[12,13] thus, there is a concern on how the phosphate groups in the lipid membrane facilitate peptide translocation into the membrane. It has also been observed that the interaction of Arg and Lys amino acids with phosphate groups of the plasma membrane plays a fundamental role in many physiological processes, such as the voltage gating of potassium ion channels[43−47] and a peptide spanning a lipid

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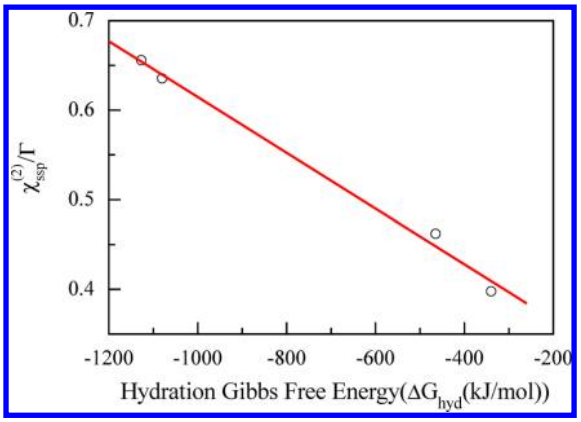

Figure 6. Fitted amplitude of $\chi^{(2)}_{ssp}$ (1655 cm^{-1}) of the ssp spectra shown in Figure 5 is plotted as a function of ion hydration Gibbs free energy. The fitting curve (red line) is used for guiding the reader's eyes and does not have any physical meaning.

bilayer. To clarify this issue, we then investigated the interaction between MP and the DMTAP and DMDAP lipid bilayers in phosphate buffer and KCl salt solution. DMTAP and DMDAP bilayers have the same hydrophobic length with DMPC bilayer, but do not contain phosphate groups. DMTAP lipid is positively charged, while DMDAP is neutral. Figure 7 shows the ssp spectra of MP molecules (with concentration of 30 μg/mL) after interacting with DMTAP and DMDAP lipid bilayers in the presence of DI water, phosphate buffer, and KCl solution with the ion strength of ~100 mM and pH of ~7.0. Similar to the results of DMPC bilayer,[14] MP can interact with DMTAP and DMDAP bilayers and form an α-helical structure in phosphate solution. However, in KCl solution, MP can interact only with DMTAP and DMPC bilayers. DMTAP and DMPC both have a positively charged choline group. This finding may suggest that the interaction between the positively charged choline group and kosmotropic anions could be an important step for effective peptide translocation into the cell membrane.

4. CONCLUSION

A systematic study on the interaction of MP with the lipid bilayers with different lipid chain lengths has been performed to examine the hydrophobic matching principle in aqueous salt environment. It is found that the hydrophobic matching is the dominant driving force for the association of MP with lipid bilayer in DI water environment. However, in kosmotropic ion environment, the ion's hydration can overcome the hydrophobic mismatching effects, leading to the insertion of MP into a lipid bilayer with a much longer hydrophobic length. When the membrane hydrophobic thickness is much larger than that of the MP's hydrophobic length, MP diffuses on a single monolayer, rather than spanning the bilayer to prevent the exposure of hydrophilic segments of peptide to the lipid hydrophobic moiety. In addition, the interaction between MP and the DMTAP and DMDAP lipid bilayers may suggest that the interaction between the positively charged choline group of the lipid and the kosmotropic ions could be an important step for effective peptide translocation into the cell membrane.

■ ASSOCIATED CONTENT

Supporting Information

Details about SFG data analysis procedures and the ssp and ppp spectra of MP amide I signal in DLPC, DPPC and DSPC bilayers in different buffer concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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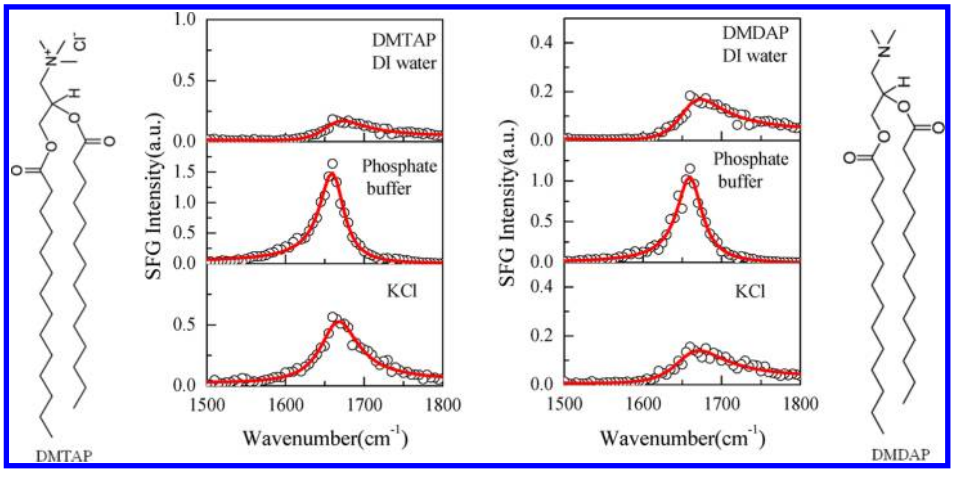

Figure 7. Ssp SFG spectra of MP molecules (with concentration of 30 μg/mL) when interacting with lipid bilayers in the presence of DI water, phosphate buffer, and KCl solution with the ion strength of ~100 mM and pH of ~7.0. Left: DMTAP; Right: DMDAP.

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