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Structural Study of Acidic Phospholipid−Basic Homopolypeptide Complexes by ^{31}P and ^{13}C Solid-State NMR Chemical Shifts

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A solid-state nuclear magnetic resonance (NMR) study for the structural elucidation of acidic phospholipid−basic polypeptide complex powder samples is presented. ^{13}C cross-polarization magic angle spinning (CP/MAS) experiments of poly(L-lysine)/dipalmitoylphosphatidic acid (DPPA) complex and poly(L-arginine)/DPPA complex powders were performed. The NMR spectra suggest that poly(L-lysine) forms a β-sheet conformation on the surface of the DPPA membrane, whereas poly(L-arginine) forms an α-helix conformation. Further, changes in the chemical shift tensors of the ^{31}P nucleus associated with the complex formation were observed, allowing us to depict the geometry of the DPPA headgroup.

1. Introduction

The interactions of membrane with proteins play an important role in living cells. Particularly, we need to understand the mechanism of the membrane−protein interaction because some membrane proteins require specific phospholipids in order to activate themselves.[1−3] As one of the typical model systems to examine such a interaction, acidic phospholipids and basic polypeptides have often been used. Poly(L-lysine)(pLYS) and poly(L-argine)(pARG) are representative basic polypeptides, and each amino acid residue of the polypeptides has a positive charge in the side chain at physiological pH. Therefore, the polypeptides are expected to electrostatically bind to negatively charged acidic phospholipids.

So far, it has gradually been understood that such a binding leads to a change in the thermotropic property of the membrane.[4] According to Ohki et al., additions of pLYS and pARG to dipalmitoylphosphatidic acid (DPPA) cause the phase transition temperature from gel (L'_β phase) to liquid crystal (L_α) to move to about 9 °C higher and about 18 °C lower, respectively, than the transition temperature (50 °C) of DPPA without these polypeptides. Here, we must note that the transition temperature would vary depending upon the amount and molecular weight of polypeptide and the pH.[5] The thermotropic behavior in the case of the pLYS/DPPA complex has been also interpreted by X-ray diffraction, and it has been concluded that the behavior is attributed to the fact that pLYS adopts a β-sheet conformation on the surface of the DPPA bilayers and strengthens the packing structure of the membrane.[6] Any X-ray crystallographic studies for the pARG/DPPA complex are not available at this moment.

Solid-state NMR spectroscopy has been thought of as one of the powerful methods for addressing structural analyses or dynamic properties of biological membranes. Although the nuclear magnetic dipolar coupling interaction allows one to obtain distances between nuclei, namely, molecular structure, it is not neccessarily easy for one to obtain the geometry of molecules because isotopic labeling of samples would be often required to detect the dipolar coupling interaction. On the other hand, since chemical shift analyses are much easier to perform than detection of dipolar coupling, it has the possibility to be a complementary methodology for structural study of molecules if structure−chemical shift (S/C) correlation[7,8] is established.

In this article, we shall determine the secondary structures of pLYS and pARG in the complex samples with DPPA by ^{13}C cross-polarization[9] and magic angle spinning (CP/MAS) NMR experiments.[10,11] Further, we also correlate the chemical shift tensors obtained by ^{31}P CP/MAS NMR experiments with the membrane−polypeptide complex formation. These results will give an interpretation of the changes in the L'_β−L_α phase transition temperature caused by the addition of polypeptides.

2. Materials and Methods

Materials. Dipalmitoylphosphatidic acid (DPPA) sodium salt, poly(L-lysine)·HCl (M_W = 30 000−70 000), and poly(L-arginine)·HCl (M_W = 15 000−70 000) were purchased from Sigma Chemical Co. (St.Louis, MO). All the materials were used without further purification. 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from NACALAI TESQUE, Inc. (Kyoto, Japan). All the samples used in this article were prepared by the following procedures.

DPPA dispersions were dissolved in 50 mM HEPES (pH 7.5) buffer solution with 23.6 mM NaOH and 76.4 mM NaCl so that the lipid concentration should be 2% (w/w). After the dispersion was incubated at 80 °C for 2 h, it was sonicated for 1 min by an ultrasonic disruptor (UR-200P, TOMY SEIKO Co., Tokyo, Japan) and was then incubated at 80 °C for 5 min and was sonicated for 10 min again in order to form small unilamellar vesicles (SUV). The dispersions were mixed with pLYS solution of which the molar ratio of the amino acid

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monomer unit/DPPA should be 1.25:1 (mol/mol). Prior to the mixing, the polypeptide was dissolved in the same amount of the HEPES buffer used above. A large quantity of precipitate was obtained by three heating−cooling cycles for the mixed dispersion (heating to 90 °C; cooling to room temperature). The precipiate of the DPPA/pLYS complex was collected by centrifugation at 9000 × *g* for 15 min. The precipitate collected was stored at 4 °C for 4 days and then lyophilized. This protocol was employed for the preparation of pARG/DPPA.

The DPPA and DPPA/HEPES samples for the controlled experiments were prepared by the following: A desired amount of DPPA was dispersed in water or HEPES buffer so that the lipid concentration should be 1% (w/w). The dispersions were incubated at 80 °C for 2 h and then cooled to room temperature. The samples obtained were stored at 4 °C for 4 days and then lyophilized.

Experimental Methods. All the experiments were performed on a JEOL GSX270 FT NMR spectrometer equipped with the MAS accessory. The intensity of the external magnetic field was 6.34 T, corresponding to the resonance frequency of 270 MHz for the ^{1}H channel. The typical ^{1}H, ^{31}P, and ^{13}C 90° pulse widths were 5.0, 5.3, and 4.7 μs, respectively. The CP contact time was 2.0 ms. The principal values of chemical shift tensors were determined from spinning side band intensities by the method of Fenzke et al.,[12] which is an extended version of Herzfeld−Berger analysis.[13]

3. Results and Discussion

We will describe the results obtained from ^{13}C CP/MAS experiments. Parts a−c of Figure 1 show the ^{13}C NMR spectra for DPPA and DPPA/HEPES and the chemical structure of DPPA, respectively. Signal assignments were performed in the manner shown in Figure 1 ($\delta(CH_3) = 15.2$, $\delta(CH_2, \gamma\text{-gauche effect}) = 25.3$, $\delta(CH_2) = 33.8$, $\delta(CH_2{-}O) = 64.5$, and $\delta(CH{-}O) = 72.3$ ppm). Here we should note that no signals appear over the range from 50 to 60 ppm for the DPPA sample. From Figure 1b, we observed that the signals derived from HEPES appeared at 52.8, 55.2, and 59.1 ppm when we used the HEPES buffer without NaOH and NaCl. Parts a and b of Figure 2 show the ^{13}C CP/MAS spectra for the pLYS/DPPA and the pARG/DPPA complex samples, respectively. From Figure 1b, and 2a,b, it is found that the signals appearing in the region from 50 to 60 ppm are derived from the C_α-carbons in the polypeptides rather than the carbons in HEPES. Additionally, we could not observe any distinguishable dependences of molecular weight distributions on the NMR spectra.

Shoji et al.[15] and Kricheldorf et al.[16] have already demonstrated that the isotropic chemical shift for the L-Lys C_α-carbon in pLYS homopolypeptide is 51.4 ppm for a β-sheet and 58.2 ppm for an α-helix structure, and the shift for the L-Arg C_α-carbon in pARG is 58.0 ppm for an α-helix structure. There are no chemical shift values available for pARG with a β-sheet structure. Under the consideration of the information about conformation-dependent chemical shifts, the signal at 53.2 ppm derived from the pLYS/DPPA complex in Figure 2 was assigned as the C_α-carbon in a β-sheet pLYS. The result is consistent with the X-ray diffraction study.[6] Ohki et al. have reported that the addition of pARG to DPPA causes the depression of the $L'_\beta{-}L_\alpha$ phase transition temperature. However, the mechanism for the thermal behavior has never been elucidated, since the structure of the pARG/DPPA complex has never been determined by X-ray diffraction because of weak and broadened diffraction patterns. From Figure 2b, we found that pARG takes an α-helix structure on the surface of DPPA.

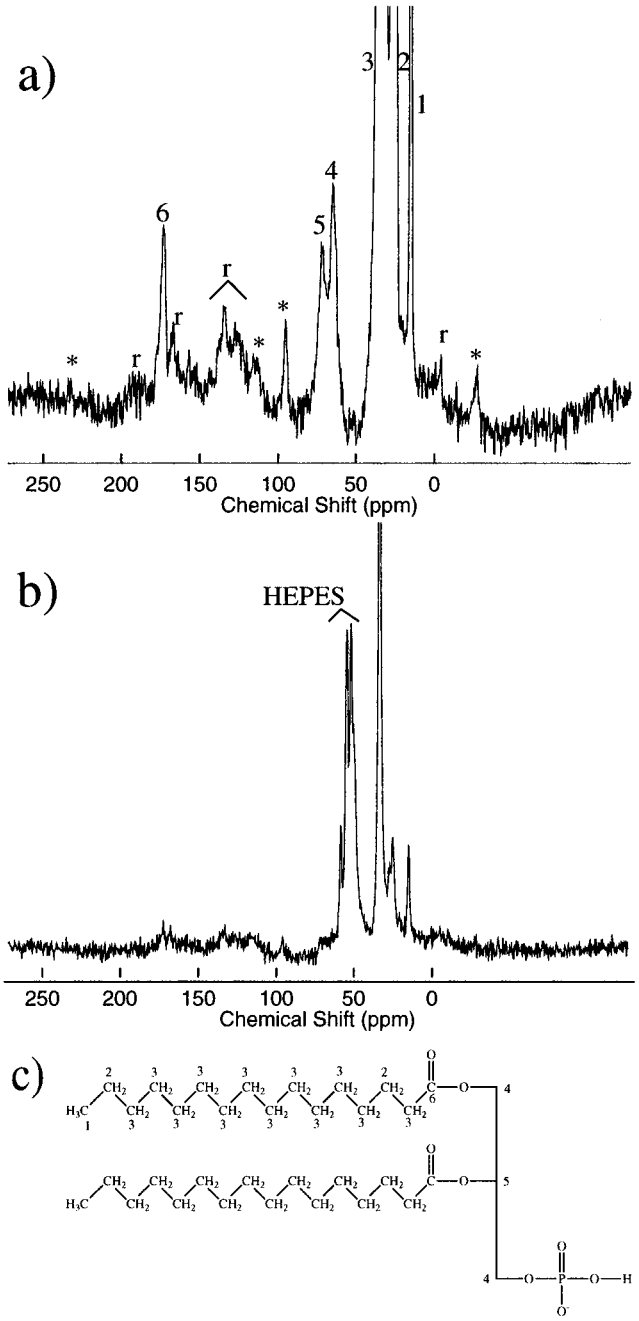

Figure 1. ^{13}C CP/MAS spectra for dipalmitoylphosphatidic acid (DPPA) (a) and DPPA/HEPES (b). The spinning speed was $\omega_r/2\pi = 4.17$ kHz. (c) The structure of dipalmitoylphosphatidic acid (DPPA) with the numberings at the signals in (a): the isotropic chemical shifts for the methyl carbon at 15.2 ppm, for the methylene carbon with γ-gauche effect at 25.3 ppm, for the methylene carbon without γ-gauche effect at 33.8 ppm, for the $H_2{-}C{-}O$ carbon at 64.5 ppm, for the $H{-}C{-}O$ carbon at 72.3 ppm, and for the carbonyl carbon at 173.3 ppm, respectively. The spinning side bands and the background signals from the rotor cap are labeled with asterisks and "r", respectively.

Next, we will describe the results obtained from ^{31}P CP/MAS experiments. The ^{31}P NMR spectra for DPPA, the pLYS/DPPA, and the pARG/DPPA complex sample are shown in parts a−c of Figure 3, respectively. From Figure 3, we can assume that the headgroup of DPPA is located in a rigid lattice at room temperature because the patterns of spinning side bands are not axially symmetric, which means that molecular rotations around the molecular axis are frozen for the lyophilized samples. On the basis of spinning side band intensities, the principal values of the chemical shift tensors for each sample were determined

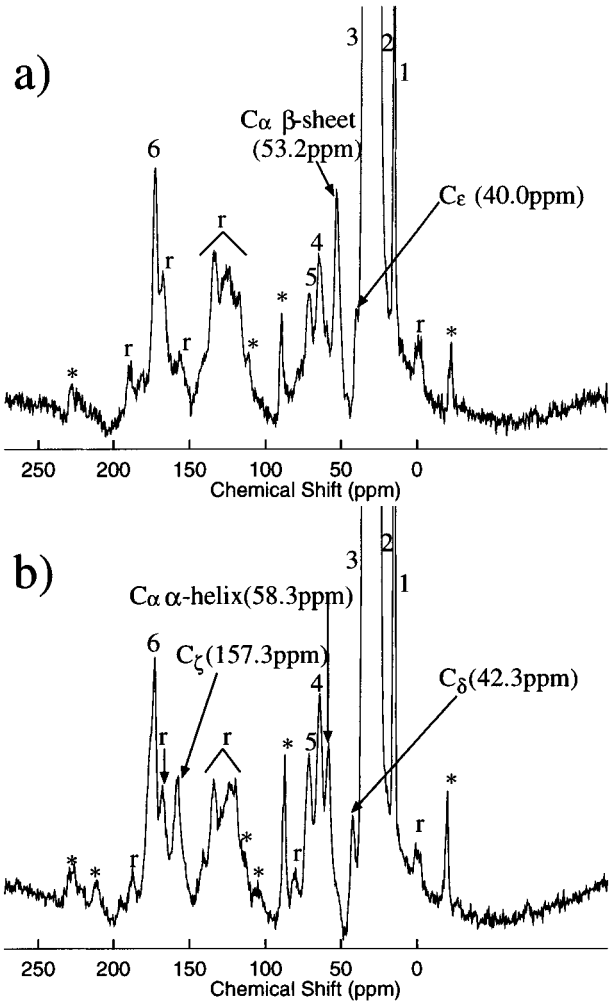

Figure 2. ^{13}C CP/MAS spectra for the pLYS/DPPA (a) and the pARG/DPPA complex (b). The spinning speeds were set at $\omega_r/2\pi = 3.80$ and 3.64 kHz, respectively. The differences can be seen at the region from 50 to 60 ppm, where the signals for the C_α-carbon in the polypeptides appeared.

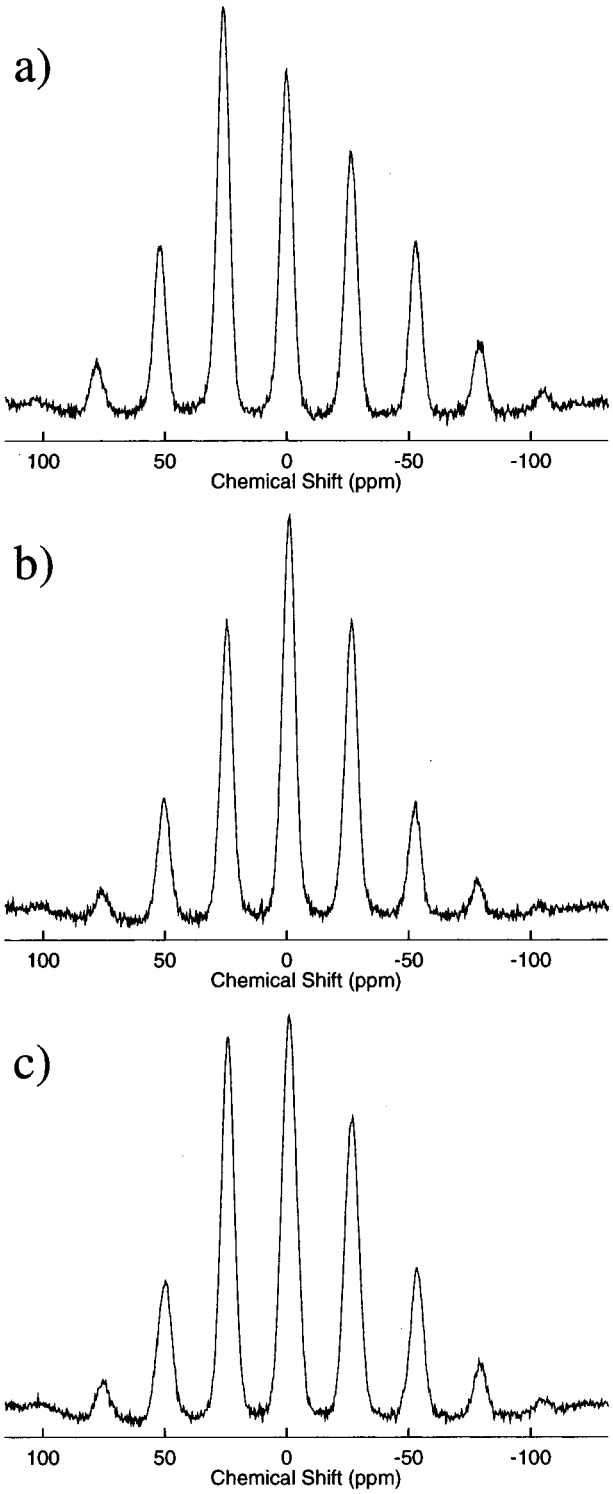

Figure 3. The ^{31}P CP/MAS spectra for DPPA (a), pLYS/DPPA (b), and pARG/DPPA (c). The spinning speed was set at $\omega_r/2\pi = 2.89$, 2.82, and 2.82 kHz, respectively. The principal values of the chemical shift tensors are summerized in Table 1.

by Herzfeld−Berger analysis[13] and are summarized in Table 1 where the isotropic chemical shift of DPPA is defined as 0 ppm. The principal values obtained from these samples suggest that the membrane−polypeptide complex formation causes the remarkable decrease of the chemical shift anisotropy (CSA),[17] particularly for the pLYS/DPPA complex sample. Turner et al.[18] have already shown that the chemical shift anisotropy regarding phosphorylated compounds can be related to the P−O bond length and to the deviation of the O−P−O bond angle from the tetrahedral value, which means that the chemical shift of the phosphorus nucleus principally depends on the geometry of the phosphate group moiety. We found that the δ_{11} and δ_{22} components are shifted to lower frequency, while the δ_{33} component is shifted to higher frequency when the phospholipid forms the complex with the polypeptide. Therefore, we need to discuss each chemical shift component in order to obtain the structural information about the headgroup of the phospholipid. Olivieri has already demonstrated S/C correlation regarding phosphorylated compounds.[19] According to Olivieri, we can make the assumption that the orientation of the chemical shift tensor of DPPA with respect to the molecular frame be the same as that of the phosphodiester model shown in Figure 4.

The electrostatic interaction between a $P-O^{(3)}$ (or $P-O^{(4)}$) oxygen and a N−H hydrogen would bring about both expansion of the $P-O^{(3)}$ (or $P-O^{(4)}$) bond distance and decrease of the $O^{(3)}-P-O^{(4)}$ angle. This is because the $O^{(3)}\cdots O^{(4)}$ electrostatic repulsion is reduced if the $P-O^{(3)}$ (or $P-O^{(4)}$) bond takes the intermolecular electrostatic interaction. The changes in electronic structure make the δ_{11} and δ_{22} components shielded. At the same time, it makes the δ_{33} component deshielded. Therefore, the experimental results suggest that pLYS binds to DPPA more strongly than pARG.

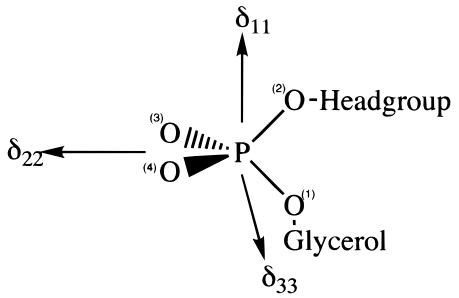

Figure 4. Expected orientation of ^{31}P chemical shift tensor with respect to the molecular fixed frame of the phosphate group. The least-shielded component of the chemical shift tensor, δ_{11}, is perpendicular to the membrane surface, and δ_{22} and δ_{33} are parallel to the surface.

TABLE 1: Principal Values of the ^{31}P Chemical Shift Tensor in ppm[a]

compd	δ_{iso}	δ_{11}	δ_{22}	δ_{33}
DPPA	0.0	66.7	12.6	−79.3
poly(L-Lys)/DPPA	−1.2	61.9	−1.2	−64.3
poly(L-Arg)/DPPA	−1.0	62.4	6.1	−71.5

[a] The isotropic chemical shift of DPPA is defined as 0 ppm.

4. Conclusions

By the ^{13}C CP/MAS experiments, we determined the secondary structure of the polypeptide binding to the surface of DPPA membrane on the basis of conformation-dependent chemical shift. For the pLYS/DPPA complex, we found that pLYS forms a β-sheet conformation on the surface of the DPPA membrane. This result is consistent with the X-ray diffraction study. We also determined the secondary structure of the pARG on the surface of DPPA membrane, which has never determined by X-ray diffraction before. The S/C correlation analysis of the C_α-carbon in pARG suggests that the main chain of the polypeptide forms an α-helix structure in contrast with the case of pLYS. Under the consideration of the ^{13}C spectra for pLYS and pARG, the conformation of the polypeptide is thought to affect the packing structure of the membrane. Further, we found that principal values of the ^{31}P chemical shift tensor change drastically with complex formation by the ^{31}P CP/MAS experiments, particularly for the pLYS/DPPA complex. This suggests that the geometry of the DPPA headgroup changes more drastically by complex formation with pLYS than with pARG. pLYS would play a role to strengthen the packing of the phospholipids by an electrostatic interaction from the negatively charged headgroup to the positively charged amino acid residue, resulting in the elevation of the $L'_\beta - L_\alpha$ phase transition temperature.

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