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Interactions of Membrane and PMAP-23 Studied by ^{31}P solid-state NMR Spectroscopy

Siwon Kim and Suhkmann Kim*

Department of Chemistry and Chemistry Institute for Functional Materials,
Pusan National University, Busan 609-735, Korea
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Abstract : ^{31}P powder pattern spectra were measured to investigate the aspects of the interaction between the MLV (Multilamellar vesicle) and PMAP-23, a membrane of cathelicidin family and then CSAs(chemical shift anisotropy) were calculated to indentify the extent of perturbation of phospholipid mobility by the peptides. We found that acidic phospholipid interacts strongly with PMAP-23, and the analogues which modified to increase the amphipathic property showed that larger change of CSA. The analogue which introduced positive charge showed the same effects with amphipathic property.
Keywords : NMR, Solid-sate NMR, antimicrobial peptide, peptide membrane interaction

INTRODUCTION

PMAP-23 is an antimicrobial peptide, a member of cathelicidin family, which known as nonspecific host defense and innate immunity.[1-4] This peptide is effective against a broad spectrum of targets, including bacteria, fungi, parasites, and even tumor cells.[5, 6] PMAP-23 is known to be membrane-active molecule that kill microorganisms through membrane disruption with no hemolytic activity.[7] But we still hardly understand about the mechanisms of the structural change and kinetics when the PMAP-23 interacts with membrane, especially the topological change of the membrane. ^{31}P Solid-sate NMR

*To whom correspondence should be addressed. E-mail : suhkmann@pusan.ac.kr

powder pattern spectrum is good to monitor the topology and phase change of the phospholipid vesicles for the mimics of biological membranes.[8]

In this work, we observed the change of CSA (chemical shift anisotropy) from the powder pattern spectrum of phospholipid MLV with and without PMAP-23 and its analogues. The analogues were designed to improve the amphipathic property and/or introduce the positive charge at the end of the sequence or middle of hydrophilic center.

EXPERIMENTAL

Design of analogues

PMAP-23 has two helical regions which separated by PXXP hinge motif at the central position. The central PXXP motif known that has an important role to penetrate into the membrane[9], so that we kept the hinge motif for all the analogues. Analogue 1 added amino group instead of carboxyl end to increase the positive head group. Analogue 2 was mutated as [^{5}L, ^{10}R] → [^{5}R, ^{10}L] to increase to change amphipathic property. Analogue 3 was mutated as [^{4}D] → [^{4}K] to change the negative charge to positive charge for the same reasons with analogue 1. Analogue 4 was included all the change of analogue 1, 2, and 3.

Sample preparation

The wild type and analogues were synthesized by classical solid phase synthetic method, then purified by FPLC (Amersham Pharmacia Biotech, Sweden) and confirmed the purity using HPLC. The molecular weight of all the peptides was confirmed by MALDI-TOF mass spectroscopy (Shimadzu, Japan). DPPC or DPPC/DPPG (1:1) was solved into the chloroform and transferred to the 4mm glass NMR tube and dried using N_2 gas stream and dried under vacuum overnight. The lipid film was suspended in 10mM Tris buffer with and without peptide and followed ten cycles of freeze and thaw.

NMR measurement and calculate CSA

^{31}P solid-state powder pattern spectrum was measured using 9.4 Tesla *unityINOVA* wide bore NMR spectroscopy (Varian USA) operating at a ^{31}P NMR frequency of 162.082MHz with nano probe to reduce sample volume. The spectra were measured with difference of phase-transition temperature caused by the peptides. CSA was calculated directly from the spectra. When the $\sigma_{//}$ shoulder reduced and unclear, the CSA was calculated by $CSA = 3|\sigma_{\perp} - \sigma_{iso}|$ where σ_{iso} is isotropic chemical shift.[10]

RESULTS AND DISCUSSION

Table 1 show the sequences of PMAP-23 wild type and its analogues which explained at experiments. ^{31}P powder pattern NMR spectra of the DPPC with PMAP-23 wild type and analogue 4 at 35℃ were showed in Fig. 1(a). When the wild type was added the spectrum showed almost the same with DPPC single component liposome, while the analogue 4 cause the change of spectrum and Fig. 2 show the CSA was decreased. Analogue 1 showed similar result with wild type and analogue 2 and 3 showed similar result with analogue 4 (data not shown). ^{31}P powder pattern spectra of the DPPC/DPPG binary mixture with and without peptide at 35℃ were showed in Figure 1 (b). Wild type and analogue 4 showed same interaction intensity with the DPPC/DPPG binary system but the CSA was largely decreased compare with the DPPC single component. Analogue 1, 2 and 3 show the same result with wild type (data not shown).

Table 1. Amino acid sequences of PMAP-23 wild type and its analogues used in this study.

peptide	*Sequence*
Wild type	RIIDL LWRVR RPQKP KFVTV WVR
Analogue 1	RIIDL LWRVR RPQKP KFVTV WVR-***NH2***
Analogue 2	RIID***R*** LWRV***L*** RPQKP KFVTV WVR
Analogue 3	RII***K***L LWRVR RPQKP KFVTV WVR
Analogue 4	RII***KR*** LWRV***L*** RPQKP KFVTV WVR-***NH2***

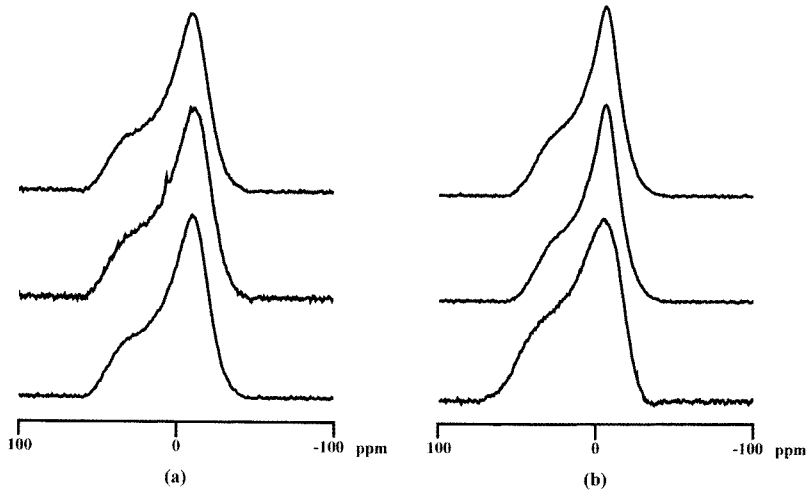

Fig. 1 ^{31}P solid-state NMR spectra of DPPC single component (a) and DPPC/DPPG binary mixture (b) MLV at 35 ℃ are showed. The bottom: without peptide; middle: with PMAP-23 wild type; upper: with analogue 4.

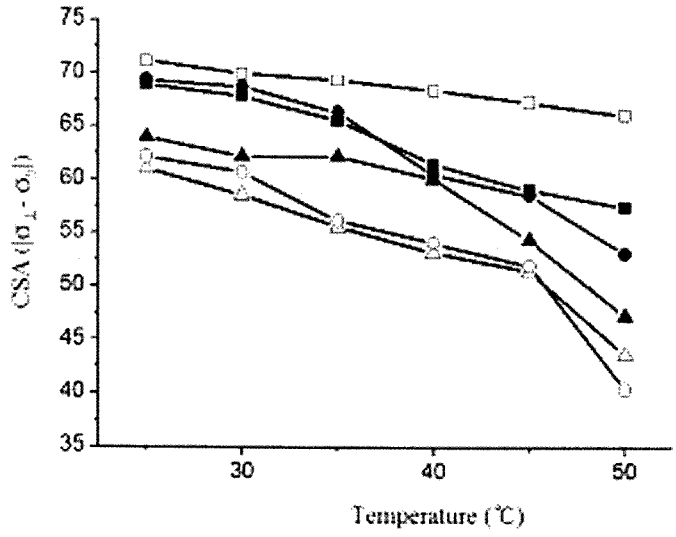

Fig. 2 Chemical shift anisotropy changes depending on the temperature from 25℃ to 50℃. Filled symbol: DPPC single component; open symbol: DPPC/DPPG binary mixture (1:1), square: lipid alone; triangle: with PMAP-23 wild type; circle: with analogue 4.

CONCLUSIONS

The CSA changes by the membrane-peptide interaction were investigated using ^{31}P solid-sate NMR study. The CSA were largely changed by the binding of analogue 4 with DPPC single component membrane. The DPPC/DPPG binary component showed different interaction aspect, the change was increased and wild type and analogues interact with the same intensity.

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