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Syndecan-4 cytoplasmic domain could disturb the multilamellar vesicle

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Abstract : Syndecan-4 cytoplasmic domain was tested to confirm the interactions with the bilayer membrane using ^{31}P solid-state NMR measurements. Syndecan-4 was known as a coreceptor with integrins in the cell adhesion. The syndecan-4 V region is not understood of its functional roles and tested its ability of the interaction with multilamellar vesicles. The ^{31}P powder pattern was dramatically changed and showed isotropic peak which imply the bilayer membrane changed its topology to the micelle-like structure. Especially, phosphatidylcholine membrane was affected this effect more than phosphatidylethanolamine membrane.
Keywords: solid-state NMR, powder pattern, membrane topology, protein-membrane interactions, Syndecan, signal transduction

INTRODUCTION

The syndecan is the major family of trans-membrane heparin sulfate proteoglycan. Four members were found in mammals which 1, 2, and 3 have a restricted tissue distribution; the syndecan-4 is located everywhere. The syndecan-4 is core protein with an integrin in the cell adhesion which mediated by cell surface receptors trigger signal transduction cascades[1-4]. Syndecan-4 comprises with four compartments. Extracellular domain can bind with ligand and accept the signal from outer world using its glycoside chains. Trans-membrane domain provide anchor for proper protein location in the cell surface membrane. Cytoplasmic domain can be dividing to three compartments, C1, V, and C2. C1 and C2 have a functional role to bind with other protein and regulate its own

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activity[5]. But there is no information for the role of the V region except known that phosphatidylinositol-4,5,-bisphosphate (PIP2) interacts with this V region[6,7].

^{31}P powder pattern is so sensitive with the topology and phase transition of the bilayer membrane[8,9]. To monitor the interactions between syndecan-4 cytoplasmic domain and multilamellar vesicle, ^{31}P solid-state NMR was utilized and analyzed its powder pattern spectrum and chemical shift anisotropy (CSA) was calculated[10].

EXPERIMENTALS

Design and synthesis of cytoplasmic peptide

Syndecan-4 V region comprise by ten amino acids sequence but we added N- and C-terminal three residues from C1 and C2 domain, respectively. The sixteen residues peptide fragment was synthesized with traditional solid-phase synthetic method[11], then purified and confirmed its purity by reverse-phase liquid chromatography using XBridge BEH130 PREP C18 column on a Waters system 600 model. This purification was achieved by equilibrating the column with 0.1% TFA in water and developing with a linear gradient of acetonitrile. The molecular weight of the peptide was confirmed by LC/MS/MS API2000 (Applied Bio System (USA)).

Sample preparation

We prepared two types of binary multilamellar vesicle; DPPC/DPPG and DPPE/DPPG to examine structural effects of lipid head group. All the phospholipids were purchased from Avanti. Dried lipid films were hydrated using Tris buffer (pH = 7.4) with and without various concentrations of peptide and followed ten cycles of freeze-and-thaw.[12,13]

NMR measurement and CSA calculation

^{31}P solid-state powder pattern spectrum were measured using 9.4 Tesla UNITY INOVA wide bore NMR spectroscopy (Varian, USA) operating at a ^{31}P NMR frequency of 162.082 MHz with nano-probe (Varian, USA) to reduce sample volume and enhance signal

sensitivity.[12] The spectrum was measured from 25 ℃ to 50 ℃ with 5 ℃ interval to check the change of transition temperature for the given phospholipid. CSA was calculated from the spectra with the difference of $\sigma_{//}$ and $\sigma_{\perp}$. When the shoulder was severely reduced and unreadable, the CSA was calculated by next equation where σ_{iso} is isotropic chemical shift.

$$CSA = 3|\sigma_{\perp} - \sigma_{iso}|$$

RESULTS and DISCUSSION

The sequence of the syndecan-4 cytoplasmic V fragment (Syn4V16) is comprises as SYDLGKKPIYKKAPTN. ^{31}P powder pattern spectrum of DPPC/DPPG (1:1) binary system at 30 ℃ and 50 ℃ was showed in Figure 1. Increasing of the peptide concentration produces isotropic peak at the center of the spectrum. This means that the topology of the bilayer membrane changed to the micelle-like or small vesicle structure. Particularly, at the high concentration of the peptide, the bilayer component was turned to the micelle-like structure. This property is so similar with that the antimicrobial peptide destroys the bacterial membrane. For this reason, we tested DPPE/DPPG (1:1) binary system with the same peptide and the results were showed in Figure 2. DPPE/DPPG binary membrane did not show the isotropic peak, which imply syndecan-4 cytoplasmic fragment interacts with PC component more strong way. This result could be explained as the interaction between DPPE and DPPG is stronger than DPPC and DPPG will make difficult to the phospholipid interacts with the syndecan peptide in the DPPE/DPPG binary membrane. The more hydrophobic head group in the PC can disturb the interaction with neighbor phosphate group. Figure 3 shows that the change of chemical shift anisotropy which will give the information about the dynamics of bilayer membrane. The change of CSA for the DPPC/DPPG is larger than for the DPPE/DPPG which shows the same tendency with the formation of the isotropic peak.

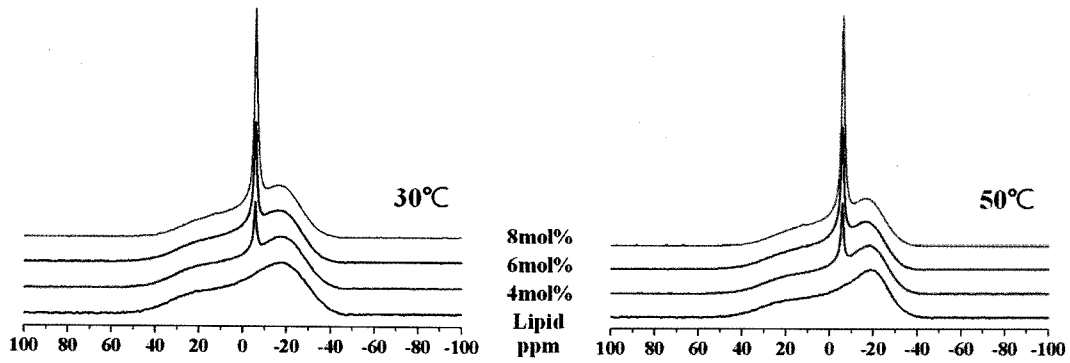

Fig. 1. ^{31}P solid-state NMR spectra for the DPPC/DPPG (1:1) binary system are showed. Left panel shows the spectrum at 30 ℃ and right panel was measured at 50 ℃, respectively. The bottom line shows the lipid membrane only and the other spectra were measured with peptide for each specified concentrations in the figure.

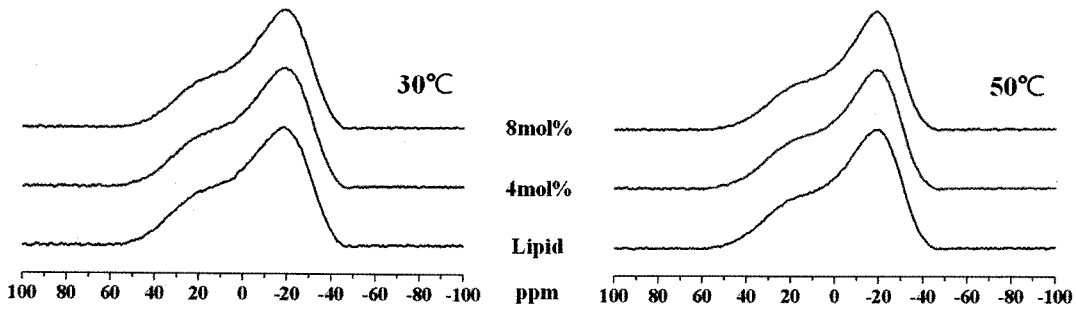

Fig. 2. ^{31}P solid-state NMR spectra for the DPPE/DPPG (1:1) binary system are showed. Left panel shows the spectrum at 30 ℃ and right panel was measured at 50 ℃, respectively. The spectrum was measured with and without peptide.

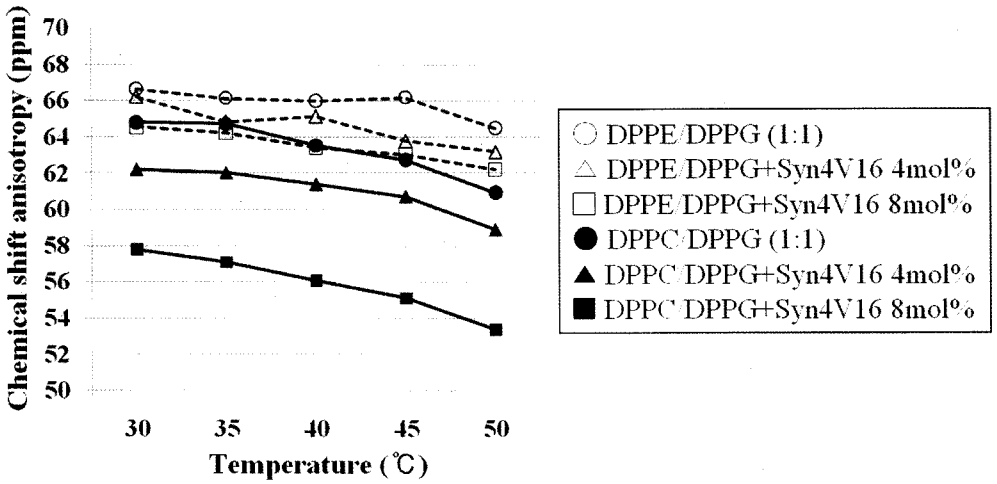

Fig. 3. Calculated chemical shift anisotropies from the figure 1 and 2 are showed for each temperature. Dotted line shows DPPE/DPPG (1:1) binary system and solid line shows DPPC/DPPG (1:1) binary system.

CONCLUSIONS

In this study, cytoplasmic domain of the syndecan-4 binds and disrupts the bilayer membrane and this phenomenon happened only with DPPC/DPPG binary membrane system. This result is unusual because many positively charged peptides interact with the bacterial membrane which major component is PE. We are using these peptides as an antimicrobial reagent. But the cytoplasmic fragment of syndecan-4 worked to the PC component which the major component of the mammalian cell membrane. From those results, we could conclude that cytoplasmic variable domain could disturb the mammalian cell membrane and this will have a specific functional role for the signal transduction pathways of the cell itself.

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