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Structure Determination of Syndecan-4 Transmembrane Domain using PISA Wheel Pattern and Molecular Dynamics simulation

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Abstract Human transmembrane proteins (hTMPs) are closely related to transport, channel formation, signaling, cell to cell interaction, so they are the crucial target of modern medicinal drugs. In order to study the structure and function of these hTMPs, it is important to prepare reasonable amounts of proteins. However, their preparation is seriously difficult and time-consuming due to insufficient yields and low solubility of hTMPs. We tried to produce large amounts of Syndecan-4 transmembrane domain (Syd4-TM) that is related to the healing wounds and tumor for a long time. In this study, we performed the structure determination of Syd4-TM combining the Polarity Index at Slanted Angle (PISA) wheel pattern analysis based on ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR of expressed Syd4-TM and Molecular Dynamics (MD) simulation using Discovery Studio 3.1.

Keywords Solid-state NMR, Syndecan, Transmembrane, Membrane protein, MD simulation

Introduction

Heparan sulfate is the polysaccharide that is covalently bound with membrane-anchored protein to form diverse heparan sulfate proteoglycans (HSPGs) on cell surface.[1] Syndecan family is the one of HSPG and has four members of Syndecan-1, Syndecan-2, Syndecan-3 and Syndecan-4. They regulate cell-to-cell interaction, cell adhesion and cell proliferation and help in healing wounds by activation of growth factor.[2,3] Syndecan family have three segments consist of an ecto-domain, a transmembrane domain and a cytoplasmic domain. The transmembrane domain and cytoplasmic domain are highly conserved. It is known that Syndecan-4 enhances focal adhesion and reduces cell motility. These functions are activated by a cytoplasmic domain that is bound with a activated protein kinase C-α (PKC-α) and phosphatidylinositol 4,5-biphosphate (PIP_2). The transmembrane domain mediates this signal to ecto-domain.[4,5] Also, it was reported that Syndecan-4 had been overexpressed on the cell surface during development of tumor.[6,7] However, the role of Syndecan-4 has not identified clearly in tumorigenesis. To understand these behaviors of Syndecan-4, it will be necessary to identify the structure of Syndecan-4. The structure of cytoplasmic domain was studied by solution NMR spectroscopy, but the structure of transmembrane domain has not been known yet due to experimental limitation like hydrophobicity and low yield of

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expression.[8]

The three dimensional structures of protein have been investigated by various methods, such as X-ray crystallography, solution NMR spectroscopy, solid-state NMR, and computational modeling. Solid-state NMR is powerful technique to characterize a transmembrane protein which needs heterogeneous environment, such as cell membrane and protein. Here, we will show one of the methods to solve the three dimensional structure of transmembrane proteins like Syd4-TM. PISA wheel pattern analysis based on ^{15}N-^{1}H 2D solid-state NMR spectra of transmembrane proteins was used to get the information of three dimensional structure in membrane environments experimentally. And we also use Molecular Dynamics simulation to get the three dimensional structure of Syndecan-4 transmembrane region (Syd4-TM) theoretically. Finally, we obtain the three dimensional structure of Syd4-TM to combine the results of PISA wheel pattern analysis and MD simulation.

Experimental Methods

2D SAMPI-4 solid-state NMR and PISA wheel pattern analysis- Uniformly ^{15}N labeled Syd4-TM expressed in membrane environment was prepared with bicelle composed of long- and short-chain ether-linked phospholipids (14-O-PC/6-O-PC, q=3.2). ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectrum was measured using 800 MHz Bruker Avance II spectrometer (Bruker Biospin, Germany) and home-built 800 MHz ^{15}N-^{1}H solid-state NMR probe with 5 mm strip shielded solenoidal rf coil. All experimental data will be published in the near future.

Polarity index at slanted angle (PISA) wheel pattern of Syd4-TM was calculated using MATLAB (MathWorks, USA).[9,10] The PISA wheel calculation was performed using structure information of an ideal α-helix. The chemical shift tensor was used with δ_{11} = 64, δ_{22} = 77 and δ_{33} = 222 for ^{15}N and δ_{11} = 3, δ_{22} = 8 and δ_{33} = 17 for ^{1}H. Initial average torsion angle of Syd4-TM was applied to Φ = -65° and Ψ = -40°. The torsion angles are important factor to determine the backbone inter helical hydrogen bonding pattern in protein. The final torsion angles were determined to Φ = -61° and Ψ = -45° undergoing optimization procedure using ^{15}N-^{1}H 2D SAMPI-4 spectrum.

Molecular Dynamics simulation of Syd4-TM- The structure of Syd4-TM with 25 amino acid sequence in membrane was created using ideal α-helix template in Discovery studio 3.1 (Accelrys, USA). The initial structure of Syd4-TM in vacuum was calculated and minimized energetically using CHARMM (c35b5) with all atom parameter of 27. This method relaxes the initial conformation through the geometry optimization. The generalized Born with a simple switching function (GBSW) model was used for implicit solvation model. The GBSW implicit model is the one of methods which calculates the electrostatic solvation energy using molecular surface with a smooth dielectric boundary. Initial position and orientation in membrane were calculated by using an oriented molecule protocol that was optimized using stepwise search for orientation and position on membrane bilayer normal. Realistic models in membrane were generated by molecular dynamics (MD) simulation. The NVT (constant-temperature and constant-volume) statistical ensemble model was used for thermodynamic ensemble. And the leap-frog Verlet was used as a dynamics integrator that was performed using a time step of 2 fs to 1 ns at 300 K, saving coordinates every 1 ps. SHAKE constraint algorithm that is constraint routine to remove the fastest degree of freedom for bond containing hydrogen was used for all simulation.

The membrane builder module in CHARMM-GUI (http://www.charmm-gui.org/) was used to draw membrane with Syd4-TM.[11] The ion placing of this system was performed by Monte-Carlo method in 0.15 M KCl. The coordination in membrane was based on the structure of Syd4-TM in MD simulation. The results of membrane builder module in CHARMM-GUI were visualized by Discovery studio 3.1.

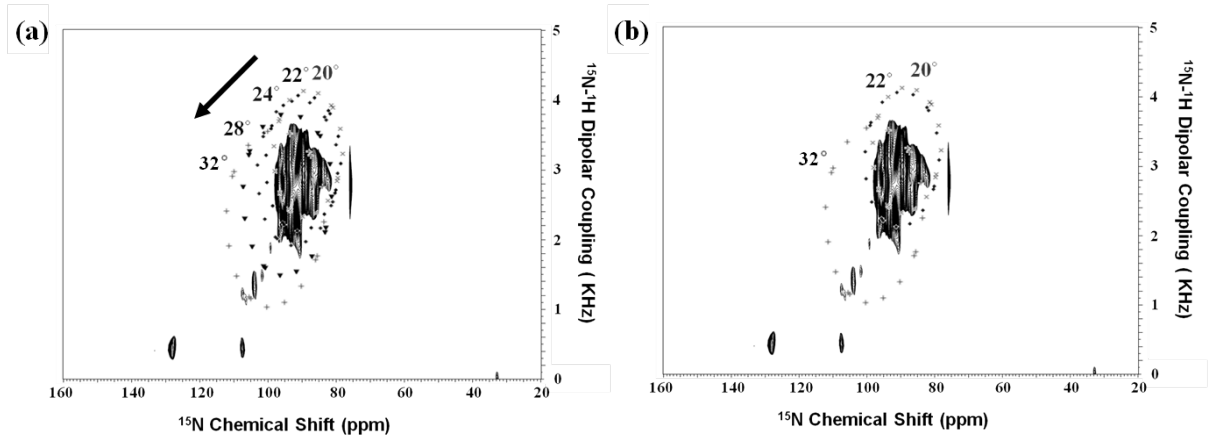

Figure 1. (a) ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectra of wt-Syd4-TM and PISA wheel pattern optimization (b) ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectra of wt-Syd4-TM with optimized PISA wheel pattern.

Results

The ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectrum of Syd4-TM oriented in membrane was obtained with "wheel-like" patterns in figure 1. This spectrum was fitted by using PISA wheel pattern analysis as shown figure 1 (a). The PISA wheel pattern is seen to be simple but they have very important structural information. The ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectrum is constructed with ^{15}N-^{1}H dipolar coupling and ^{15}N chemical shift of amides of Syd4-TM protein. These spectral parameters have alignment information of amides axes relative to the direction of applied external magnetic field. PISA wheel pattern of Syd4-TM based on ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectrum was obtained by optimization process of tilt (or slant) angle of transmembrane helix in figure 1 (a) and analyzed to have curved α-helix segment of two different angles in the transmembrane domain. This means that Syd4-TM in membrane-like environments such as bicelle has two helical axes (32°, 21±1°). In other words, Syd4-TM has a curved α-helical structure in bilayer membrane.

The three dimensional structure of Syd4-TM was also calculated with computer simulation using Discovery Studio 3.1. Energy minimization in vacuum, initial conformation and position of Syd4-TM in membrane were calculated by CHARMM (c35b5). Figure 2 shows the structure of Syd4-TM after energy minimization and membrane application process with implicit membrane using CHARMM (c35b5). The structure of Syd4-TM which has a slightly curved structure was embedded in hydrophobic region of membrane. The membrane thickness was 23 Å that is consistent with membrane thickness of bicelle.

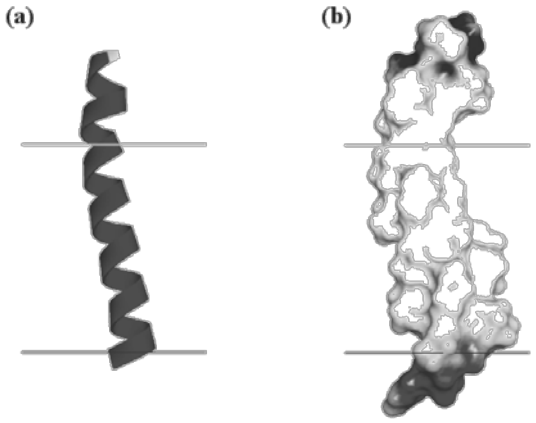

Figure 2. Structure of Syd4-TM after energy minimization and membrane application process in implicit membrane using CHARMM (c35b5). (a) Ribbon model of Syd4-TM in membrane (membrane thickness = 23 Å). The two helical axes were shown in this structure. (b) Electrostatic potential surface model of Syd4-TM in membrane. This model shows more curved

structure than ribbon model

which Newton's equations of motion are united for

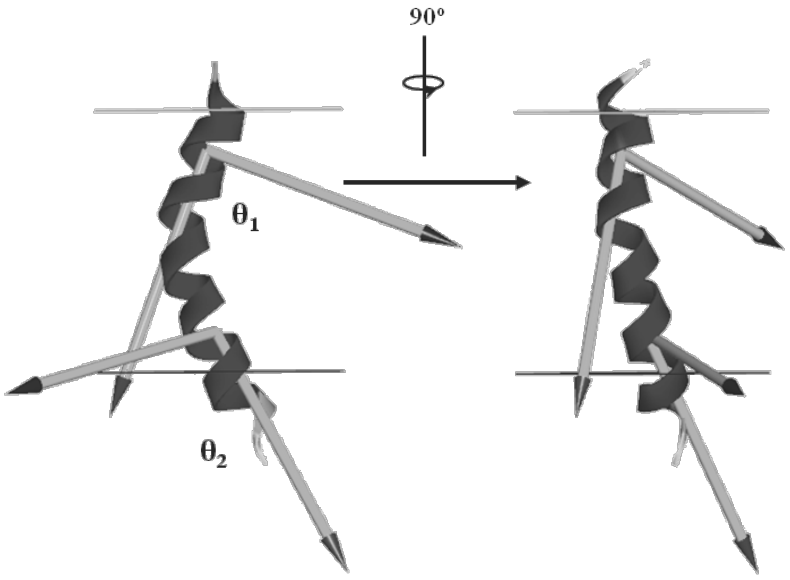

Figure 3. The structure of Syd4-TM based on Molecular Dynamics simulation. Tilt angles θ_1 and θ_2 are 20.7° and 31.5°, respectively. The average dihedral angle of this conformer were Φ = -65° and ψ = -38°.

The realistic models in membrane were then generated by Molecular Dynamics simulation with the NVT statistical ensemble model and the leap-frog Verlet of a dynamics integrator. MD simulations are carried out using a classical mechanics method in all atoms in the protein system.

The best conformer of Syd4-TM in MD simulation is shown in figure 3 and tilt angles θ_1 and θ_2 are 20.7° and 31.5°, respectively. The average dihedral angle of this conformer were Φ = -65° and ψ = -38°. They are very similar to dihedral angles that were

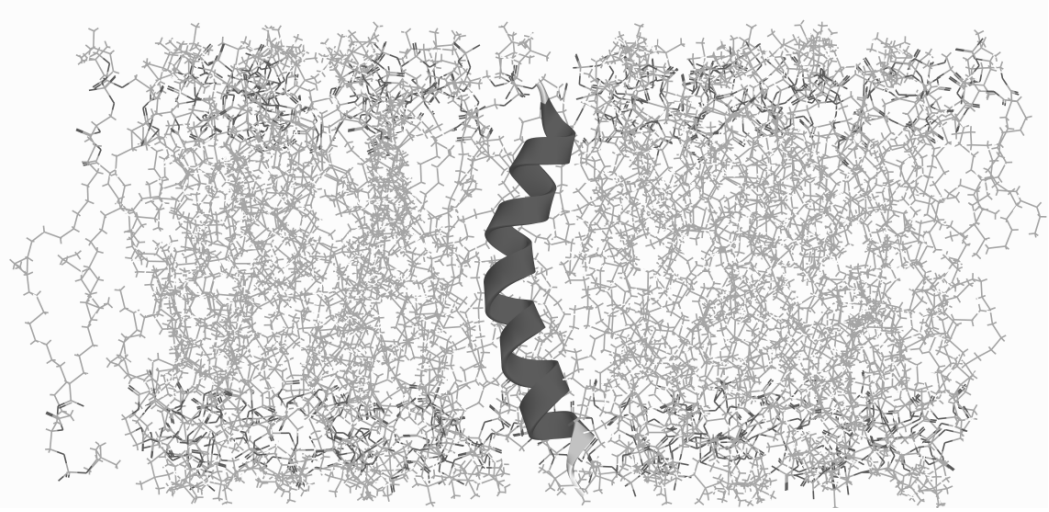

Figure 4. The structure of Syd4-TM in membrane by using membrane builder module in CHARMM-GUI

optimized by PISA wheel pattern analysis (The dihedral angle of ideal α-helix: Φ = -60° and ψ = -45°). This conformer is well-matched with the PISA wheel pattern analysis based on ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR spectrum.

The best conformer obtained from the MD simulation combined with lipid bilayer complex using membrane builder model in CHARMM-GUI is shown in figure 4. It was shown that a 25 amino acid region of Syd4-TM was well-placed in lipid bilayer membrane.

Discussion

Although membrane proteins are closely related to the important biological functions and related diseases, the structures of membrane proteins, especially transmembrane domains, were not fully understood due to the experimental limitations such as insufficient yields and low solubility.

In this study, we have combined PISA-wheel pattern analysis based on ^{15}N-^{1}H 2D SAMPI-4 solid state NMR spectra and the computational modeling using Discovery studio 3.1 in order to determine the 3D structure of transmembrane protein in membrane environments.

The results are well matched to show that the Syndecan4-TM in membrane-like environments such as bicelle has two helical axes (32°, 21±1°) which mean the Syndecan4-TM has a curved α-helical structure in bilayer membrane.

Acknowledgements

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