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Structural Change in Transmembrane Region of Syndecan-4 by Mutation

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Abstract Transmembrane(TM) proteins 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 TM proteins, 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 TM proteins.

We tried to produce large amounts of Syndecan-4 containing TM domain(SDC4-TM) that is related to the wound healing and tumor. Also, mutated SDC4-TM was studied to investigate structural change by modification of dimerization motif. We performed the structure determination by the Polarity Index at Slanted Angle (PISA) wheel pattern analysis based on ^{15}N-^{1}H 2D SAMPI-4 solid-state NMR of SDC4-TM and computational modeling using Discovery Studio 2016.

Keywords Solid-state NMR, Syndecan, Transmembrane, Membrane protein, Computational modeling

Introduction

Syndecans are transmembrane heparan sulfate proteoglycans by covalently bound with heparin sulfate which performs the most important function for survival of organism in biological cell membranes, such as cell-to-cell interaction, cell adhesion, cell proliferation and helps in healing wounds by activation of growth factor.[1-3]

Syndecan-4(SDC4) in gene family of four syndecans has distinct responses from others in tumor progression and metastasis: SDC4 is overexpressed by tumor progression.[4-13] The mechanism of SDC4 in tumorigenesis have not clearly revealed, but two facts were reported that overexpression of SDC4 is stimulated by tumor suppression molecule and reverted anti-adhesion effect by tenascin-C that prevent the cell adhesion and enhance the tumor proliferation. Therefore, the overexpression of SDC4 in tumor cell could prevent proliferation of tumor cell.[14-15]

SDC4 is composed of three parts in cell membrane, extracellular(ecto) domain, transmembrane(TM) domain, and cytoplasmic(cyto) domain and known to form a dimeric structure by GxxxG motif.[16] The functions of SDC4 are occurred by the interaction between ecto-domain and various factors, eg. growth factor and matrix molecules. These functions in ecto-domain are regulated by cyto-domain that interacted to actin cytoskeleton and signaling molecules, such as phosphatidylinositol-4,5-

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bisphosphate. The communication between cyto- and ecto-domain is mediated by the structural change of TM domain.[17] Thus, the structural determination of TM domain is essential to understand the function of SDC4.

The 3D structures of proteins have been studied generally using X-ray crystallography, solution nuclear magnetic resonance (NMR) spectroscopy. However, it has the limitation due to their perplexing properties and environments. TM proteins are highly insoluble and very difficult to crystallize and stabilize based on detergents, and also requires the membrane-mimetic system to investigate their native structure.

Solid-state NMR(SSNMR) spectroscopy is the best solution for the above mentioned limitations. This technique has a unique capability to investigate the native structures of TM proteins in membrane-like environments. Magnetically aligned bicelle is good medium for structural study of TM protein in SSNMR spectroscopy, and the SAMPI-4 technique, one of separate local field spectroscopy, provides simplified orientational information for TM proteins by using analysis of Polarity Index at Slanted Angle (PISA) wheel pattern.[18-20] The three-dimensional structures of TM proteins based on SSNMR spectroscopy and PISA wheel pattern analysis could be confirmed and ratified by using computational calculation methods.

The computational calculation has been developed to discover three-dimensional structures of proteins during the past half century. The homology modeling and refinement methods are used to predict the protein structures with reasonable accuracy. However, it is difficult to predict the structure of TM protein since they have lack of structural database.

In this study, we introduced computational calculation based on SSNMR spectroscopy for the structural study of SDC4s containing TM domain. Prior to structural study of SDC4 in the membrane, the dimeric properties of SDC4 were investigated in dodecylphosphocholine(DPC) micelle by using solution NMR spectroscopy. The structural information for SDC4, tilt angle, and average dihedral angle, were obtained by analysis of PISA wheel pattern for SDC4. The final structure of SDC4 was confirmed by computation modeling. The resulting structures from two methods, SSNMR study and computation modeling, were mutually consistent.

Experimental Methods

Sample preparation- To investigate the structure of SDC4, the sample for NMR spectroscopy was prepared to TM domain of SDC4(wt-SDC4-TM, VLAAL IVGGV VGILF AVFLI LLLVY). Also, mutated G8L:G12L SDC4-TM(mSDC4-TM, VLAAL IV**L**GV V**L**ILF AVFLI LLLVY) was prepared to investigate the structural modification by mutation of GxxxG motif.[21] Wt- and mSDC4s were prepared to uniformly ^{15}N labeling for NMR spectroscopy. We already reported successful expression and purification of SDC4s containing TM domain.[22]

NMR spectroscopy for SDC4 series- SDC4s for solution NMR spectroscopy were prepared with micelle. Final concentration of SDC4s was 1 mM with 0.1 M DPC-d_{38} micelle in 300 μl H_2O/D_2O (90%/10%) at pH 4.0. The ^{1}H-^{15}N HSQC spectra for SDC4 were measured using triple resonance indirect detection probe with the XYZ-gradient unit at 800 MHz Bruker Avance II spectrometer(Bruker Biospin, Germany). The H_2O resonance was suppressed by 'water flip back' sequence.

SDC4s for SSNMR spectroscopy were prepared with bicelle that composed of long- and short-chain ether-linked phospholipids (14-O-PC/6-O-PC). This bicelle was made to q=3.2. The SSNMR spectra for SDC4 were measured by ^{15}N 1D CP-MOIST(the Cross-Polarization with Mismatch-Optimized IS Transfer) and ^{15}N-^{1}H 2D SAMPI-4 experiments at 800 MHz Bruker Avance II spectrometer and home-built ^{15}N-^{1}H 800 MHz SSNMR probe with 5 mm strip shielded solenoidal rf coil.[25-26]

The PISA wheel pattern for SDC4s was calculated by using MATLAB (MathWorks, USA).[27-28] In this calculation, the chemical shift tensors were used with $\delta_{11} = 64$, $\delta_{22} = 77$ and $\delta_{33} = 222$ for ^{15}N and $\delta_{11} = 3$,

δ_{22} = 8 and δ_{33} = 17 for ^{1}H respectively. The average torsion angle at initial calculation applied to Φ = -65° and Ψ = - 40°. This structural information is important factor to establish the inter-helical hydrogen bonding pattern for protein backbone. Finally, the optimized torsion angle was determined to Φ = -61° and Ψ = - 45° through the optimization process.

Computational calculation of SDC4 series- All modeling and calculations in this study were performed to protocols in Discovery studio 2016 (Biovia, USA). We already reported the monomer structure of wt-SDC4-TM that constructed by using energy minimization and MD simulation because it was represented to have low sequence identity with homologous protein.[29] In this study, we introduced homology modeling with multiple sequence alignment to solve the problem for low identity. Their homologous proteins were searched by PSI-BLAST with PAM30 scoring matrices. These were used to template structures for homology modeling. The sequences of SDC4s were aligned to template proteins by using multiple sequence alignment method: 3J5P, 1ECN and 1IFK for wt-SDC4-TM, and 4BWZ, 3J5P and 2LK9 for mSDC4-TM. Finally, monomeric structures were constructed to homology modeling with results of sequence alignment. Dimeric structures of wt-SDC4-TM were simulated by using 'Dock proteins(ZDOCK)' protocol.[30] These results were scored by using a pairwise shape complementarity (PSC), as a scoring function, which is simple shape complementarity method. This function is a fundamental component of protein-protein docking scoring functions and utilizes a geometric descriptor based on the surface curvature of surface area. The GxxxG motif in SDC4s was used to constraints for the binding site in this simulation. The dimeric structure of mSDC4-TM was constructed by mutation based on the structure of wt-SDC4-TM.

The CHARMM36 all-atom empirical force fields(c39b1) was utilized to all calculation after dimerization of SDC4s.[31] Their position and orientation in relative imaginary or implicit membrane were optimized by using 'Add membrane and oriented molecules' protocol with generalized-born with simple switching(GBSW) implicit solvation model. The GBSW solvation model calculates the electrostatic solvation energy using molecular surface with a smooth dielectric boundary. Membrane thickness in this calculation was used to 23 Å. Finally, the conformations of dimeric structures in the implicit membrane were refined by using energy minimization method with SHAKE constraint algorithm.

Results

Solution NMR spectroscopy for SDC4 series- The ^{1}H-^{15}N HSQC spectrum is like a fingerprint since the cross peaks in this spectrum represent backbone amide site of protein. However, it was not matched for the wt-SDC4-TM as expected, as shown figure 1. The cross peaks were represented to 53 peaks, therefore, the structure of wt-SDC4-TM in this spectrum has asymmetry dimer structure, as already reported.[22]

The mSDC4-TM in HSQC spectrum was represented to have cross peaks that were corresponded with its residues. It means that mSDC4-TM was the loss of dimeric substructure by mutation of GxxxG motif, and it corresponds with previous reported.[21]

Solid state NMR spectroscopy for SDC4 series- The 1D and 2D SSNMR spectra of SDC4 were measured from magnetically aligned bicelle at 42 °C. The 1D spectra of SDC4s were obtained by using ^{15}N 1D CP-MOIST experiment cross-polarization technique that are widely used to obtain fundamental information for protein topology in bicelle sample. The ^{15}N resonances of wt-SDC4-TM and mSDC4-TM in ^{15}N 1D spectra were present from 80 to 100 ppm, as shown figure 2. It means that SDC4s were oriented parallel to lipid bilayer normal in uniaxially aligned bicelle.[32]

The SAMPI-4 spectra of SDC4 in bicelle were measured by using homebuilt ^{15}N-^{1}H SSNMR probe in 800 MHz NMR spectrometer. The SAMPI-4

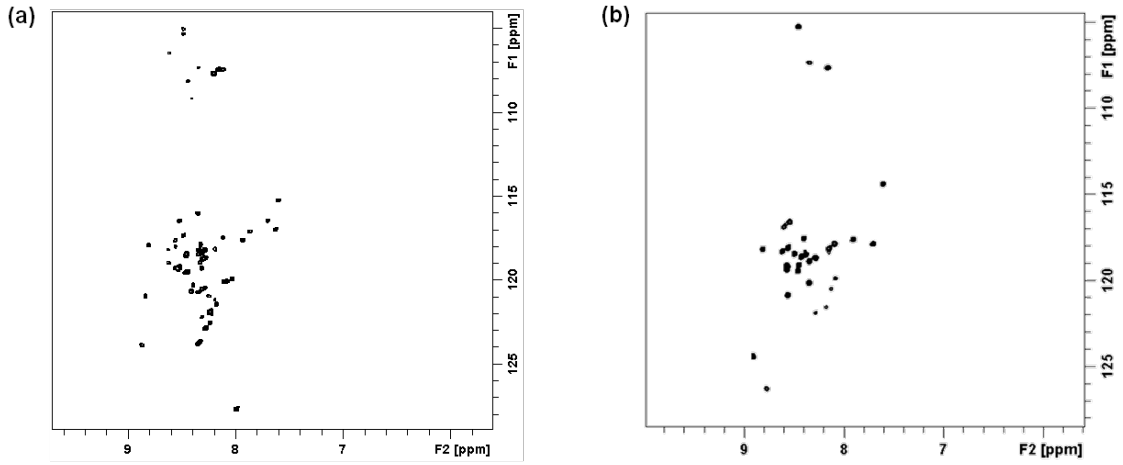

Figure 1. The HSQC spectra of uniformly ^{15}N labeled SDC4s. (a) The resonances for wt-SDC4-TM in this spectrum are almost doubled from the number of its residues. (b) The resonances for mSDC4-TM, they are corresponded with the number of residues. They were measured at Bruker Avance II 800 MHz NB NMR spectrometer.(NS=8, TD(f2)= 1024, TD(f1)=256, B_1 field strength = 23936 Hz)

SSNMR technique is a proper method to obtain important structural information, such as tilt angle and rotational diffusion rate in bicelle. This spectrum is composed of ^{1}H-^{15}N dipolar coupling at F1 domain and ^{15}N chemical shift of amides of SDC4s at F2 domain and shows "wheel-like" circular patterns if the TM protein has helical structures. The SAMPI-4 spectra of SDC4s are represented as shown in figure 3.

These patterns of SDC4s in SAMPI-4 spectra were fitted and analyzed by using PISA wheel pattern analysis as shown figure 4. It has alignment

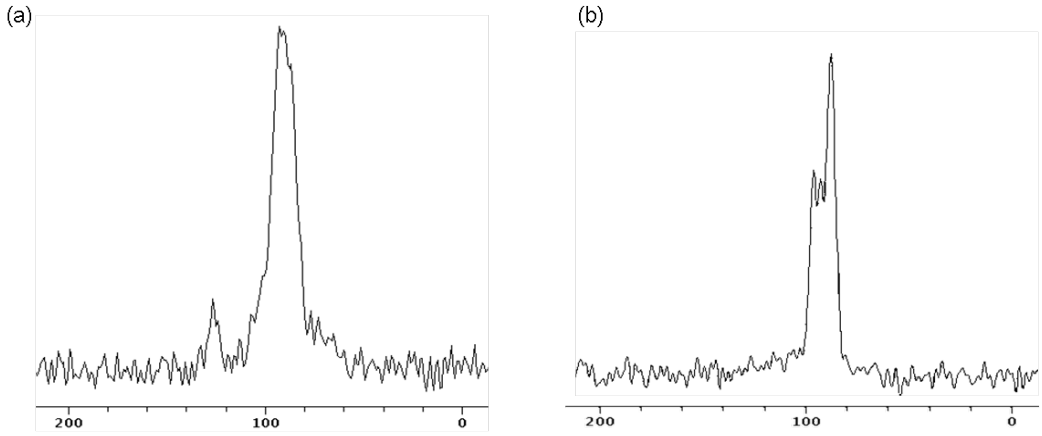

Figure 2. The ^{15}N 1D CP-MOIST spectra of (a) wt-SDC4-TM, (b) mSDC4-TM. All resonances for SDC4s are ranged from 80 to 100 ppm, it means that they are parallel for bilayer normal of 'unflipped' bicelle: It means that two SDC4s are placed in lipid membrane. They were measured at Bruker Avance II 800 MHz NB NMR spectrometer with home built 1H-15N 800 MHz solid-state NMR probe.(NS=3072, B1 field strength = 43860 Hz)

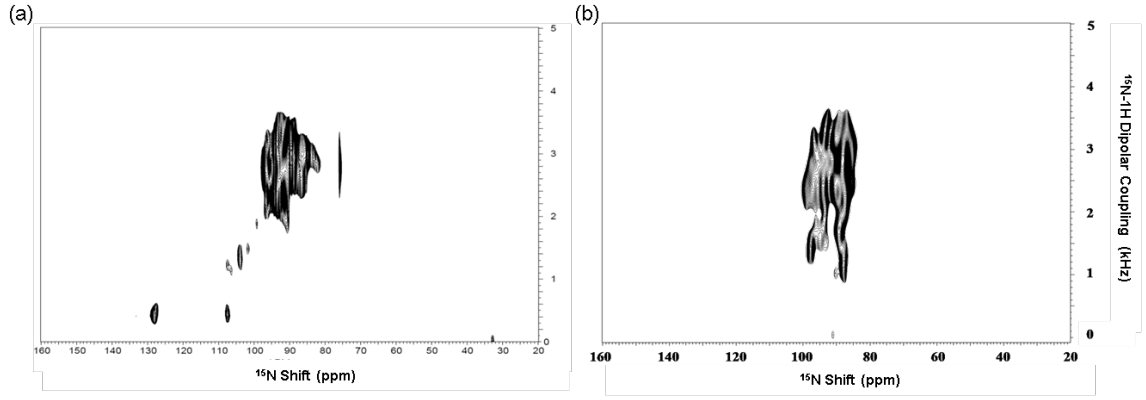

Figure 3. The ^{15}N-^{1}H 2D SAMPI4 spectra of (a) wt-SDC4-TM, (b) mSDC4-TM. ^{15}N-^{1}H dipolar coupling constants of SDC4s are measured about 2-3 kHz. These values are corresponded with coupling constants of ^{15}N-^{1}H heteronuclear interaction from lipids in 'unfilpped' bicelle under static condition. (NS=256, TD(f2) = 512, TD(f1) = 64, B_1 field strength = 43860 Hz)

information of amides axes relative to the direction of applied external magnetic field.

PISA wheel analysis based on SAMPI-4 gave information for tilt angles of SDC4s. In these results, wt-SDC-TM and mSDC4-TM were represent to have two helix axis(20°~22° and 32° for wild-type, and 22° and 34° for mutant)

Computational calculation of SDC4 series- The three-dimensional structures of wt-SDC4-TM were also calculated with computer simulation using Discovery Studio 2016. The modeled structures of wt-SDC4-TM were generated by using homology modeling with template proteins. The modeled structures of all SDC4s have a slightly curved α-helix structure. This curved structure was formed with GxxxG motif as the center because glycine residues have high conformational flexibility.

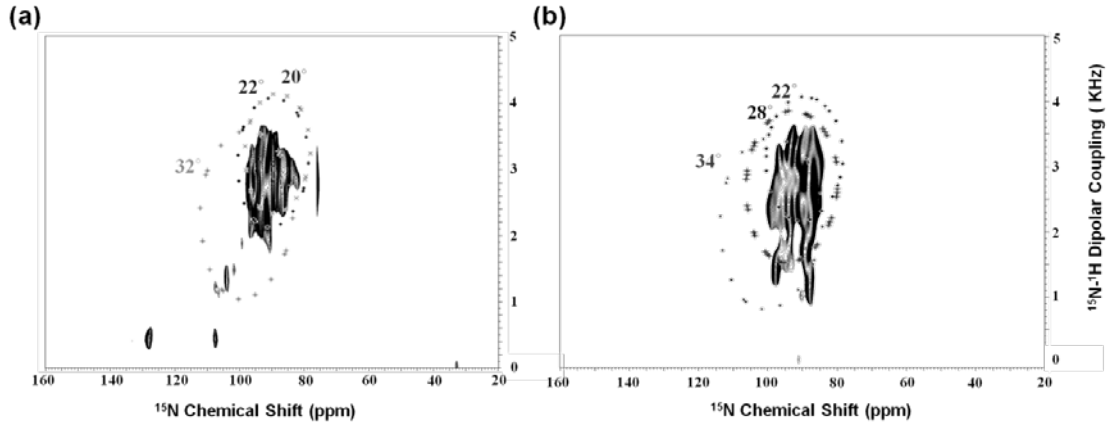

Figure 4. The PISA wheel patterns of the ^{15}N-^{1}H 2D SAMPI4 spectra of (a) wt-SDC4-TM, (b) mSDC4-TM. The tilt angles for (a) wt-SDC4-TM is about 20-22° and 32°, and for mSDC4-TM is about 22°, 28°, 34°. They were calculated by using the chemical shift tensors of ideal α-helix for Φ = -61° and Ψ = - 45°.

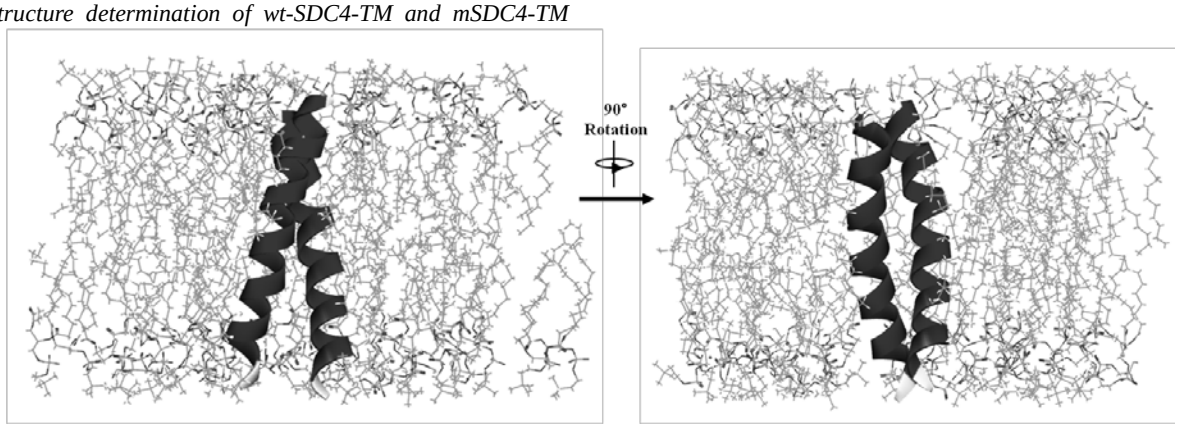

Figure 5. The dimer structure of wt-SDC4-TM in membrane environments. Dimer of wt-SDC4-TM have four tilt angles of 28.9°, 21.2°, 22.3° and 21.0°. The orientation of wt-SDC4-TM dimer was calculated by Discovery studio 2016 and protein protocols. Lipid membranes were constructed by CHARMM-GUI

The protein docking method was performed to predict the dimer structure of SDC4s. At this point, dimer complex for wt-SDC4-TM was calculated to the symmetric dimer.

The initial orientation of SDC4s in the membrane was calculated by imaginary membrane application process using CHARMM36. In this simulation, the membrane thickness was used to 23 Å that is consistent with a membrane thickness of bicelle used in SSNMR study and the SDC4s were embedded in the imaginary membrane. These dimeric structures of SDC4s in membrane applied energy minimization process to refine structures.

Finally, the dimeric structure of wt-SDC4-TM in the membrane was represented as shown figure 5. The SDC4-TM formed the dimer structure by hydrogen bond at three residues, G8 and G12 in GxxxG motif and A16. At this structure, two monomer subunits have different orientations for the membrane. Also, they had a different average dihedral angle on Ramachandran plot (was not shown): the dimeric structure of wt-SDC4-TM was determined to have asymmetric dimer structure and corresponded to that of HSQC spectrum. Two monomer subunits have different four tilt angles, 28.9° and 21.2° for subunit1 and 22.3° and 21.0° for subunit2 with respect to the bilayer normal of the imaginary membrane. Also, these tilt angles were within the scope for calculated tilt angles in PISA wheel analysis although some error remained at subunit1. The asymmetry for

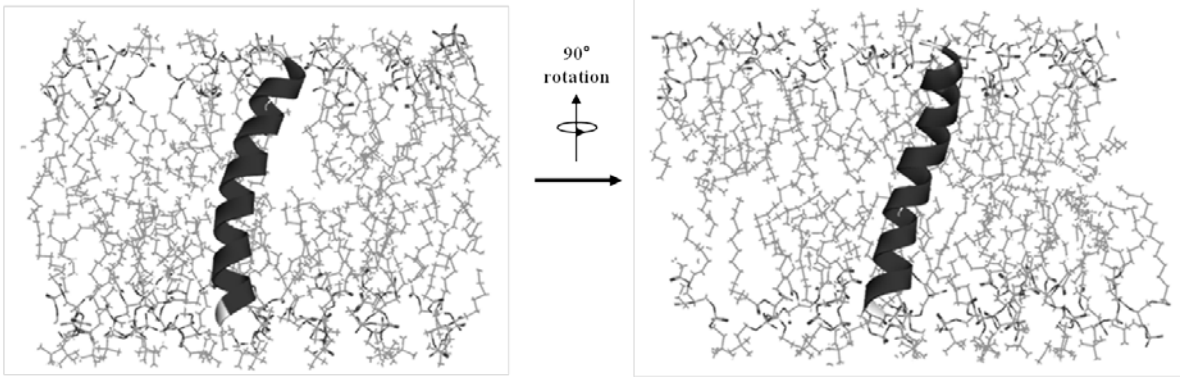

Figure 6. The structure of mSDC4-TM in membrane environments. This structure has tilt angles of 34° for N-terminal region and 34° for C-terminal region. The orientation of mSDC4-TM was calculated by Discovery studio 2016 and protein protocols like wt-SDC4-TM. Lipid membranes were constructed by CHARMM-GUI.

wt-SDC4-TM dimer was assumed to cause the formation of the cluster by small residues, L5, G8, and G9. (data not shown) This cluster has similarity to already known structure of asymmetric dimers.[33]
The mSDC4-TM, like wt-SDC4-TM, was constructed by multiple sequence alignment and homology modeling. This structure in the membrane was shown figure 6. It was obvious curved α-helix, as it does with wt-SDC4-TM. The tilt angle of mSDC4-TM appeared to 34° for the N-terminal region and 24° for the C-terminal region. It was corresponded with PISA wheel analysis.
The monomeric structure of wild-type and mutated SDC4-TM seemed to be very similar, but they could differ the center of curve and alignment of dimerization motif as shown in figure 7.
This result shows that mutation of GxxxG lead alternation of helix alignment in motif this broken dimer formation of SDC4.

Discussion

Although TM proteins are closely related to the important biological functions, they were not fully understood due to the experimental limitations such as hydrophobicity. In this study, we used PISA-wheel pattern analysis based on ^{15}N-^{1}H 2D SAMPI-4 solid state NMR spectra and the computational modeling for structure determination.
Finally, mutation of GxxxG motif in SDC4 for this study could assure to induce modification of helix alignment that breaks dimer formation of SDC4.

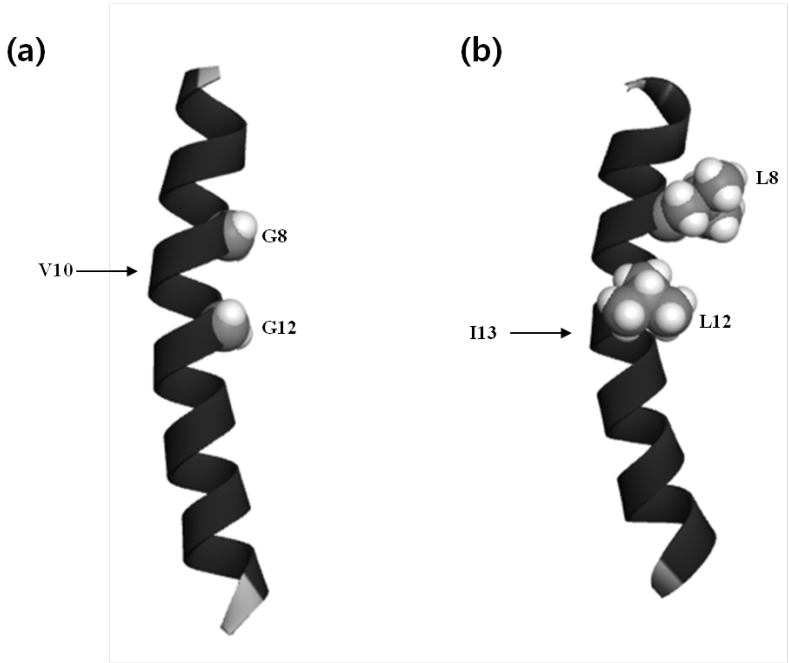

Figure 7. The monomer structures of SDC4s. In this figure, two glycines in (a)wt-SDC4-TM are similarly aligned to helical axis, but two leucine in (b)mSDC4-TM are not. And also, they are shown obvious difference in curved center of helix. Curved centers of SDC4s are V10 for wt-SDC4-TM and I13 for mSDC4-TM.

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References

1. S. E. Stringer, and J. T. Gallagher, *Int. J. Biochem. Cell Biol.* **29**, 709 (1997)
2. G. David, B. Schueren, P. Marynen, J. J. Cassiman, and H. Berghe, *J. Cell. Biol.* **118**, 961 (1992)
3. K. Elenius, and M. Kalkanen, *J. Cell Sci.* **107**, 2975 (1994)
4. D. M. Beauvais, and A. C. Rapraege, *Reprod. Biol. Endocrin.* **2**, 3 (2004)
5. M. Gulyás, and A. Hjerper, *J. Pathol.* **199**, 479 (2003)
6. T. R. Roskams, G. David, B. Damme, V. Desmet, and *J. Pathol.* **185**, 290 (1998)
7. F. Echtermeyer, M. Streit, S. Wilcox-Adelman, S. Saoncella, F. Denhez, M. Detmar, and P. F. Goetinck, *J. Clin. Invest.* **107**, R9 (2001)
8. A. Shimazu, M. A. H. Bachchu, M. Morishita, M. Noshiro, Y. Kato, and Y. Iwamoto, *J. Dent. Res.* **78**, 1791 (1999)
9. S. Yung, A. Woods, T. M. Chan, M. Davies, J. D. Williams, and J. R. Couchman, *FASEB J.* **15**, 1631 (2001)
10. A.Woods, J. R. Couchman, and Curr. Opin. *Cell Biol.* 13, 578 (2001)
11. Y. Zhang, M. Pasparakis, G. Kollias, and M. Simons, *J. Biol. Chem.* **274**, 14786 (1999)
12. F. Baba, K. Swartz, R. Buren, J. Eickhoff, Y. Zhang, W. Wolberg, and A. Friedl, *Breast Cancer Res. Tr.* **98**, 91 (2006)
13. M. E. Lendorf, T. Manon-Jensen, P. Kronqvist, H. A. B. Multhaupt, and J. R. Couchman, *J. Histochem. Cytochem.* **59**, 615 (2011)
14. W. Huang, R. Chiquet-Ehrismann, J. V. Moyano, A. Garcia-Pardo, and G. Orend, *Cancer Res.* **161**, 8586 (2001)
15. R. Chipquet-Ehrismann, P. Kalla, C. A. Pearson, K. Beck, and M. Chiquet, *Cell* **53**, 383 (1998)
16. S. Choi, E. Lee, S. Kwon, H. Park, J. Y. Yi, S. Kim, I. O. Han, Y. Yun, and E. S. Oh, *J. Biol. Chem.* **280**, 42573 (2005)
17. H. Udo, N. Kent, and P. Norbert, *Nature Rev. Mol. Cell Biol.* **6**, 530 (2005)
18. J. S. Waugh, *Proc. Natl. Acad. Sci. USA* **5**, 1394 (1976)
19. A. A. Nevzorove, and S. J. Opella, *J. Magn. Reson.* **164**, 182 (2003)
20. F. M. Marassi, and S. J. Opella, *J. Magn. Reson.* **144** 150 (2000)
21. I. C. Dew, and K. R. MacKenzie, *Proc. Natl. Acad. Sci.U.S.A.* **104** 20782 (2007)
22. T. Park, M. Lee, J. Kim, and Y. Kim, *Process Biochem.* **46** 1166 (2011)
23. J. Song, J. Kim, S. S. Choi, and Y. Kim, *Bull. Korean Chem. Soc.* **34** 3577 (2013)
24. M. H. Levitt, D. Suter, and R. R. Ernst, *J. Chem. Phys.* **84**, 4243 (1986)
25. A. A. Nevzorove, and S. J. Opella, *J. Magn. Reson.* **164**, 182 (2003)
26. F. M. Marassi, and S. J. Opella, *J. Magn. Reson.* **144**, 150 (2000)
27. J. Wang, J. Denney, C. Tian, S. Kim, Y. Mo, F. Kovacs, Z. Song, K. Nishimura, Z. Gan, R. Fu, J. R. Quine, and T. A. Cross, *J. Magn. Reson.* **144**, 162 (2000)
28. R. Chen, Z. A. Weng, *Proteins* **51**, 397 (2003)
29. S. S. Choi, J. H. Jeong, J. S. Kim, and Y. A. Kim, *J. Kor. Magn. Reson.* Soc. **18**, 58 (2014)

30. R. Best, X. Zhu, J. Shim, P. Lopes, J. Mittal, M. Feig, and A. D. Mackerell, *J. Chem. Theory & Comput.* **8**, 3257 (2012)
31. S. H. Park, A. A. Angelis, A. A. Nevzorove, C. H. Wu, and S. J. Opella, *Biophys. J.* **91**, 3032 (2006)
32. D. E. Warschawski, A. A. Arnold, M. Beaugrand, A. Gravel, É. Chartrand, and I. Marcotte, *Biochim. Biophys. Acta* **1808**, 1957 (2011)
33. J. H. Brown, *Protein Sci.* **15**, 1 (2006)