HIGH FIELD ^{19}F SOLID-STATE NMR SPECTROSCOPY FOR STRUCTURAL AND CONFORMATIONAL STUDY OF M2 MEMBRANE PROTEIN

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Introduction

The M2 protein, an integral membrane protein of Influenza A virus, forms a proton-conductive ion channel activated at low pH (*1, 2*). This 97-residue protein has a 24-residue N-terminal segment, a 54-residue C-terminal domain, and a single transmembrane domain consisting of a 19-residue α-helix (*3*). It has been documented that the His37 side chain plays an important role in the activation of the M2 H^+ channel (*2, 4*). The question is whether or not the Trp41 side chain, which is located in the transmembrane segment, plays the role of gating in the channel. The 6th position of the Trp41 indole ring was fluorinated. Since ^{19}F is very sensitive to the chemical shift environment, the ^{19}F chemical shift at high and low pHs, which correspond to the closed and open states of the channel, respectively, allows us to monitor the chemical environment of the Trp41 side chain at the different channel states.

Experimental

High-resolution ^{19}F solid-state NMR spectra were recorded at 310 K on a Bruker 600 NMR spectrometer with a Bruker H/F/X triple-resonance MAS NMR probe.

Results and Discussion

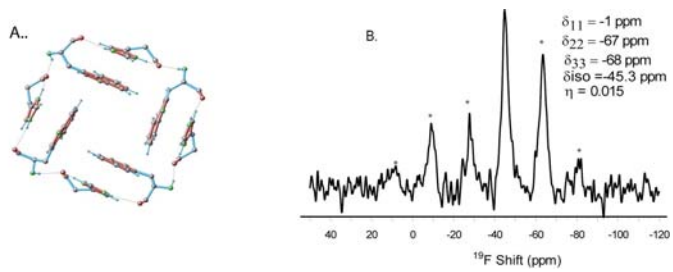

Figure 1. A). MD simulated Trp41 sidechain orientations at pH 5 (i.e. the open state) in the M2 tetrameric transmembrane domain. Only are the His37 and Trp41 residues shown in this plot. B) ^{19}F MAS NMR spectrum of 6-F-Trp41 M2 protein in DOPC/DOPG (1:4 molar ratio) at pH 5. The sample was spun at 8.0 kHz. The asterisks in the spectrum indicate the spinning sidebands. The chemical shift anisotropy was obtained by fitting the sideband intensities.

In the M2 H^+ channel, there exist repulsive interactions between the His37 and Trp41 sidechains. Therefore, upon the charge and discharge at the His37 residues such repulsive interactions will vary resulting in conformational change in the Trp41 sidechain orientations. The Trp41 sidechain orientations at pH 5.0 and 8.0 were calculated using the COSMOS calculation program (5) considering the His37 charge state. Only those residues in the transmembrane domain were considered in the calculations. Fig.1A shows the preliminary result at pH 5.0, corresponding to the open state of the channel. Fig. 1B shows the ^{19}F MAS NMR spectrum of the 6-F-Trp41 M2 protein in DOPC/DOPG (1:4 molar ratio) at pH 5.0. At high pH (i.e. 8.0), the calculated Trp41 sidechain orientations appear to close the channel (Figure not shown). It is expected that the chemical environment of the labeled fluorinated sites in the close state differ that in the open state. Related experiments are undergoing.

Acknowledgements

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References

[1] Pinto, L. H., *et al*., *Cell* ***69,*** 517-528. (1992)
[2] Okada, A., *et al*., *Biochemistry* ***40***, 6053-6060. (2001)
[3] Nishimura, *et al*., *Biochemistry* ***41***, 13170-13177. (2002)
[4] Wang, C., *et al*., *Biophysical Journal* ***69***, 1363-1371. (1995)
[5] Stenberg, U., *et al. COSMOS:Computer Simulation of Molecular Systems"-www.software.de*