Structure Study of the Full-Length M2 Proton Channel in Membrane Bilayers

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Introduction

M2 protein, being pivotal in virus pathogenicity, is a homo-tetrameric proton channel in bilayers, contains the TM and amphipathic domain (AM) with the extracellular and intracellular sequence on both terminus [1]. PISEMA (Polarization Inversion of Spin Exchange at Magic Angle) is used to provide detailed structure information of TM helix in bilayers, e.g. the tilt angle of helix and orientation of peptide planes relative to the bilayer [2]. The structure of TM-AH domain in bilayers has been found a kink at G34 in the TM domain by introducing tilt angles of 32° and 22° to bilayer [3]. The focus of this study will be on the structure of full-length M2, a native form with tightly physiological relevance and longer truncated M2(22-97).

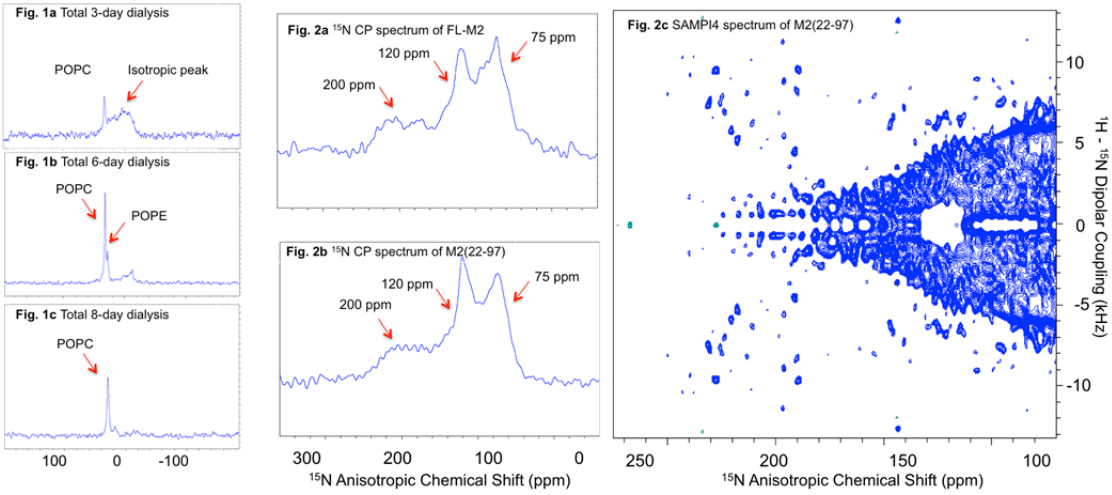
Experimental

M2 protein is purified and reconstituted into POPC: POPE bilayers by the dialysis method. ^{31}P spectra are used to verify the bilayer alignment and removal of detergents. The alignment of M2 protein in bilayers is studied by 3.2 mm Low-E static ^{1}HX probes. 1D ^{15}N CP (cross polarization) and 2D PISEMA spectrum are performed on 400 MHz Bruker 2-channel and 720 MHz 4-channel VARIAN Inova console in the NHMFL.

Results and Discussion

Given the fact of the negative curvature induced by the small headgroup of POPE, we tested the lipid alignment by using ^{31}P spectra. Figure 1 indicates that complete removal of detergent can improve the bilayer alignment. Fig 2a and b show that M2 proteins are partially aligned into POPC: POPE with some distinct frequencies observed on the 2D spectrum (shown in Fig. 2c).

Figure 1: a,b,c show the progress in reconstitution as a function of dialysis time. Figure 2 show ^{15}N spectra of (a) full length M2 and (b) M2 (22-97) in one dimensional spectra. Figure 2c shows PISEMA spectra of M2 (22-97) showing excellent alignment for a portion of the sample.



Conclusions

Our results show that POPC: POPE can serve as a mimic of native membrane system with other lipid molecules, e.g. SM and cholesterol. The distinct frequencies on the 2D spectrum suggest that at least partially, the M2 proteins can be uniformly aligned in such a bilayer composition and the aligned portion from the powder pattern/isotropic fraction can be optimized with further effort.

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References

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