M2 Proton Channel from Influenza A Virus Studied by Solid State NMR Spectroscopy

Mukesh Sharma (FSU, Chemistry and Biochemistry); Huajun Qin (FSU, Chemistry and Biochemistry); David Busath (BYU, Physiology); Timothy A. Cross (NHMFL, FSU, Chemistry and Biochemistry)

Introduction

M2 protein of influenza A virus forms a homo-tetrameric proton channel involved in modifying virion and trans-Golgi pH for virus infection and inhibited by drugs Amantadine and Rimantadine. Recently, two distinct structures were determined for the transmembrane domain of M2 protein. Stouffer et al. (1) published the crystal structure of the M2 Transmembrane domain and Schnell and Chou (2) determined the structure of M2 residues (18–60) that included the TM domain and 17 residues of the cytoplasmic tail. However, both structures differ in drug binding site and conformational rearrangement upon binding from solid state NMR studies performed in lipid bilayer (3, 4). The aim of this research is to study the correlation of structure and function of intact M2 proton channel and mechanism of inhibition by influenza drugs in lipid bilayer.

Experimental Results

In order to obtain high resolution structural information in native bilayer like environment, we have expressed and purified intact M2 protein and two peptides M2(22-46) and M2(22-62) as a maltose binding fusion protein from E. Coli membrane and reconstituted in a DMPC:DMPG(4:1) and DOPC:DOPE(4:1) lipid bilayers. Channel activity of the purified peptide was established using the proteoliposome fusion assay. In channel conductance assays we found that M2 (22-62) (1 mg peptide/8 mg DMPC + 2 mg DMPG/ml H2O, diluted to 3% in the cis chamber) forms active channels in POPE: POPC: POPS: Cholesterol planar lipid bilayers. With asymmetric KCl solutions (1 M, pH 8 cis, 0.1 M, pH 6 trans), the time-averaged membrane current was >10-fold higher using M2(22-62) proteoliposomes than with protein-free liposomes (N>8) for Vm = ±100 mV and M2 (22-46) proteoliposomes.

Conclusions

Two dimensional Solid State NMR correlation experiments performed on uniform $^{15}$N labeled and amino acid specific labeled M2 (22-62) peptide suggest four fold symmetric arrangement of transmembrane and amphipathic helices tilted at 34° and 75° with respect to lipid bilayer normal. Results from different constructs suggest that helical tilt angle of transmembrane domain in intact M2 protein (26°) is smaller compared to that of isolated peptides M2 (22-46) and M2 (22-62) and oligomeric state of the channel is stabilized due to interactions of amphipathic helices. These functional and NMR studies shed new light on correlations of structure and function for this channel.

Acknowledgements

This work was supported by NIH Grant # AI23007.

References