Conformational Analysis of the Full-Length M2 Protein and the Drug Binding in Different Constructs of Influenza M2 Protein by Solid State NMR

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The 97-residue influenza A M2 protein forms a tetrameric proton channel that is essential for the virus life cycle and is the major target for the amantadine class of antiviral drugs. Extensive structural information has been obtained about the transmembrane (TM) domain and the adjacent amphipathic helix (AH) domain; however, little is known about the N-terminal ectodomain and the C-terminal cytoplasmic tail. The proton channel is activated by the low pH of the endosome, with His37 of the TM domain being responsible for acid activation and proton conduction. Several p*K*a's of His37 have been determined in different constructs of M2. A recent study of M2(21-97), indicated that the p*K*a's of His37 are 7.1 and 5.4.

Using 2D ^{13}C solid-state NMR spectroscopy (SSNMR), we have analyzed the secondary structure and dynamics of full-length M2 (M2FL). The conformation and dynamics of M2FL are sensitive on the membrane composition. The protein exhibits strong β-strand chemical shifts for the extra-membrane residues when bound to 1,2-dimyristoyl-sn-glycero-3-phosphocoline (DMPC) bilayers, but predominantly α-helical chemical shifts in cholesterol-rich lipid membranes. Chemical shift prediction for various structural models and comparison with the experimental spectrum indicate that DMPC-bound M2FL contains a β-strand segment, which is most likely located in the N-terminal ectodomain, and a significant coil content in the rest of the two extra-membrane domains. The fact that cholesterol increases the α-helical content of the extra-membrane domains may be relevant to M2 interaction with the matrix protein M1 during virus assembly and budding.

Residue Ser31 in the TM of the M2 protein is the primary binding site for the antiviral drug amantadine (Amt) and rimantadine (Rmt). Previous studies show no chemical shift perturbation in the ^{15}N-^{13}C HETCOR spectra when Amt is titrated in the VM+-bound M2(21-61). On the contrary, M2(22-46) reconstituted in the VM+ membrane displays drug-induced chemical shift perturbation. We use SSNMR to show that the length of the construct and the membrane composition affect antiviral drug binding to the M2 channel. 2D ^{15}N-^{13}C HETCOR spectra of M2(21-97), that contains the entire cytoplasmic tail show chemical shift perturbation of residue Ser31 in both DMPC and VM+ membranes, indicating that the antiviral drug binds to the pore. The fact that Amt is not able to bind M2(21-61) is due to the extremely high membrane curvature created by the amphipathic helix alone. These results demonstrate the ability of SSNMR to determine protein-drug interaction in the presence of different lipid membranes.