

Characterizing the Tryptophan-41 Residue of Influenza M2 Protein

Introduction:

Despite the large strides in medical research and increased access to medical facilities, influenza still results in the deaths of about 36,000 people per year in the United States [1]. This has led researchers to look for alternative strategies to combat influenza. One of these strategies involves the inhibition of a crucial protein, the M2 ion channel. If the M2 ion channel can be characterized in terms of mechanism of activation, researchers can develop more molecules to inhibit M2 and/or develop other strategies to reduce M2 function. I intend to use fluorescence to study the function of a highly conserved amino acid residue, tryptophan 41, in M2 channel activation. I will undertake this project during the spring and summer of 2007 at the Pinto Laboratory at Northwestern University.

Background:

Influenza infects human cells by using hemagglutinin to recognize sialic acid on the surface of epithelial cells. Cells incorporate the virion particle through endocytosis. In an attempt to destroy the virion particle, cells lower the pH of the endosome. The lower pH of the endosome causes the activation of M2, a viral envelope protein. M2 activation results in a reduced pH within the virion, which causes the release of RNA from the viral membrane protein [2]. This RNA can then diffuse into the cell to replicate the virion particle and destroy the cell. If M2 is inactivated, there will be no reduction in pH within the virion and no release of RNA, halting further infection of influenza.

The M2 ion channel is a homotetrameric protein with each subunit contributing an α -helix towards the transmembrane pore. Two amino acid residues, histidine 37 (His-37) and tryptophan 41 (Trp-41), are highly conserved [3]. They line the pore of M2 and are critical to proper function. Not having these residues (substitution or deletion) results in unregulated proton movement through the channel [4]. Better understanding of M2 depends on determining how these two residues function in channel activation.

Currently, the two main inhibitors for the M2 protein are amantadine and rimantadine. However, these molecules only have inhibitory effects on the M2 protein of the A strain of influenza. There are three strains of influenza; A, B, C. The A strain is the most virulent and effects many species; the B strain mainly effects humans; and the C strain mainly affects a few species of horses. It has been observed that the some forms of the A strain have developed resistance to amantadine and rimantadine, such as avian flu [5]. By characterizing the two highly conserved residues within M2, other inhibitors for M2 can be developed for the A strain and lead to further understanding in developing inhibitors for the B and C strain viruses.

Literature Review:

Evidence exists that supports the conclusion that Trp-41 has a role in the gating mechanism of M2 [6]. Initially, the Trp-41 occupies a certain position in the transmembrane pore. There is a change in the pH causing activation of M2 and it is uncertain in what ways the position of the Trp-41 changes following activation. Currently, the Pinto Lab is doing studies in the transmembrane domain to determine the movement of Trp-41. I will conduct complementary studies of Trp-41 to determine its movement.

Research Questions:

I intend to create three mutants on the M2 protein. Each of these mutants will convert three C-terminal residues to cysteine residues. To the cysteine residues, I will attach fluorescent labels and observe the fluorescence of these labels under various pH. By doing so and observing the change in fluorescence due to any possible Trp-41 movement, I will be able to answer the question what role does Trp-41 have, in terms of its spatial movement, in the gating mechanism following M2 activation.

Methods:

Molecular biology will be used to induce mutagenesis within three different mutants on a residue in the C-terminal of the M2 protein. The mutagenesis will occur on a Cys minus (lacking cysteines) and single tryptophan background protein within the bacteria *E. coli*. Mutations will induce a change to cysteine in each mutant. Cysteine is desired as it has a free thiol group that can be used to attach fluorescent labels using disulfide linkages. These new cysteine mutants will then be purified from *E. coli* using nickel affinity columns and reconstituted in liposome to test for protein function *in vitro*. RNA encoding the mutants will also be made and injected into *X. laevis* oocytes to test for protein function *in vivo*. Following confirmation of proper function, the mutants will be reconstituted in liposomes and fluorescent labels will be attached to the cysteine mutants. The fluorescence of these proteins will be measured using the Keck Biophysics Facility fluorometer and tested for changes at various pH.

Preparation:

For this project I have had preparation both through classes at Northwestern and previous lab experience. I am currently finishing the 210 biology sequence. This course has taught me the basics of protein biochemistry and how to conduct proper molecular biology experiments. My lab experience has included work at the Klein Lab at Washington University in St. Louis over the past two summers; and for the past six months, I have worked in the Pinto Lab. Here I have learned proper mutagenesis techniques, fluorescence techniques and oocyte injection. This has not only supplemented the techniques necessary for completion of my project, but also my education within biology courses. Based on this I believe I have enough experience and knowledge to complete this project. I expect to turn this project into an honors thesis within the Biological Sciences department of Northwestern University.

Conclusion:

Characterizing the movement of the Trp-41 residue in the M2 protein can result in the understanding of how to disrupt of influenza infections. Currently, only amantadine and rimantadine can block the M2 protein function. By characterizing the movement of the highly conserved tryptophan residue, researchers can develop further strategies to inhibit M2 and therefore, influenza. The development of such strategies is necessary as many strains of influenza have gained drug resistance and the threat of a cross species strain of influenza increases daily.



Bibliography

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