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Mapping the Resistance Potential of Influenza's H + Channel Using an Anti-Viral Blocker

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Abstract

One of the biggest problems faced by biotechnologists and pharmacologists all over the world is the difficulty in predicting the strains of viral diseases that are coming into circulation. The problem becomes much worse when it becomes difficult to predict the virus's resistance pattern. Influenza A virus has many factors that play a role in its infection cycle. Here, we are trying to study how the virus exhibits resistance against an anti-viral blocker. The blocker that is used in this case is amino-adamantane antiviral inhibitor (adamantine). The interaction between the binding sites of Influenza M2 protein and the anti-viral blocker is visualized using simulations and this enables us to understand the concept with more clarity. The knowledge will prove to be useful during drug development and future studies in this field.

Keywords: Influenza A virus, Influenza M2 protein, adamantine,

1. Introduction

Over the past few decades, Influenza virus has caused many epidemics and pandemics throughout the world. Influenza is a negative-sense, single-stranded, segmented RNA virus. They are of three types- A, B and C. We are focusing mainly on Influenza A. Currently, H1N1 and H3N2 are the strains in circulation, in humans. Influenza is categorized on the basis of two molecules- Hemagglutinin and Neuraminidase. Hemagglutinin brings about agglutination of blood while Neuraminidase, found on the surface of influenza viruses that enables the virus to be released from the host cell. Influenza has RNA as genetic material and so they can undergo rapid mutations. This gives rise to a variety of strains which in turn creates a massive problem for scientists. They are forced to try and predict new strains that are going to come into circulation in order to try and tackle this deadly disease. Antigenic shift (Drift and Shift) is the process by which two or more strains of virus come together and combine to form a new subtype. Hence, Influenza requires the scientists to be one step ahead of the disease at all times. This unpredictability is what mainly intrigued us and convinced us to work on this topic. Research on influenza includes studies on molecular virology, how the virus produces disease (pathogenesis), host immune responses, viral genomics, and how the virus spreads (epidemiology). These studies help in developing influenza countermeasures; for example, a better understanding of the body's immune system response helps vaccine development, and a detailed picture of how influenza invades cells aids the development of antiviral drugs.

Here, in this project, we are trying to study the way in which an anti-viral blocker such as amantadine inhibits or blocks the H+ channel of Influenza. This helps us in further understanding the resistance potential of a viral strain. We have combined aspects of drug delivery with bioinformatics and 3D simulation techniques to understand the concepts better.

2. Materials and methods

NCBI database provides a large amount of data regarding genomic and biomedical sciences like research papers, protein sequences, information regarding biomolecules.

Clustal Omega was used to create the multiple sequence alignments and hence obtain the phylogenetic tree to study the evolutionary relationships between sequences.

RCSB-PDB provides a variety of tools for analysis of structure and functioning of proteins.

PubChem Compound contains a large amount of information regarding the structure and functioning of various compounds thereby helping us to study their interactions with different proteins/receptors.

RasMol is a molecular visualization tool that can be used to view protein and nucleic acid structures for analysis.

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ConSurf Server is used to map binding sites on proteins. This information can be used to study protein-ligand interactions

SPDBV Version 4.1.0 is used to analyze several proteins at the same time. The proteins can be superimposed in order to study structural similarities and compare them.

Open Babel software was used to convert file from one format to the other (for example, from .sdf to .pdb).

Hex 6.3 is a software used to study protein-ligand interactions. It is also used for molecular superposition.

Blender is a 3D creation suite. It can be used for simulations, rigging, animations etc.

Unity3d is used for visualization, modelling and animation. It has a fully functioning physics engine and scripting is very flexible. Five steps were involved in mapping the resistance potential of Influenza's H+ channel against an anti-viral blocker:

Phylogenetic Analysis

Hemagglutinin sequences of about 10 different strains of Influenza A virus was obtained from the NCBI Database. The strains selected were H1N1, H3N2, H3N8, H9N2, H7N9, H9N4, H10N5, H8N4, H11N1 and H10N2. Phylogenetic analysis was performed using Clustal Omega. This tool created the Multiple Sequence Alignment of the chosen sequences and formed the phylogenetic tree, which indicates the evolutionary relationship between the strains.

Structural Analysis

RCSB-PDB was used to obtain the M2 protein structure which was then analyzed using RasMol. RasMol also enabled us to count the number of hydrogen and di-sulphide bonds. The anti-viral blocker, Amantadine's structure and information regarding physical and chemical properties were obtained from PubChem Compounds.

Biding Site Prediction

To understand the protein-ligand interaction, it is necessary to know the binding sites on the M2 protein. Active sites were identified using ConSurf server. The results proved that Ser31 and Gly34 were the M2 binding sites. This result was parallel to that obtained through wet lab experiments.

Protein Modelling and Docking

We used SPDBV software to analyze the structure of M2 and to create the receptor model for docking. Open Babel software was used to convert the structure of Amantadine obtained from PubChem (in .sdf format) to .pdb format.

The model obtained from SPDBV was uploaded as receptor while converted amantadine structure was uploaded as ligand in Hex 6.3 to study the protein-ligand interaction (docking).

Visualization/Simulation

In order to understand the concepts better, we used two software- Blender for modelling and Unity 3D for creating simulations. Blender was used to create models of molecules and the M2 protein. It was used to design the protein and render artistic effects to the same. The modelling made use of tools like Sculpt, Scale and Rigg effects. The modelled object was exported in .fdx format and then imported on to Unity 3D where the behavior of the channels (electrostatics) was coded in the C# algorithm and the results were obtained.

3. Results and discussion

Phylogenetic Analysis

Table 1: Hemagglutinin Sequences used

Influenza A virus	GenBank ID
(A/SouthDakota/01/1991(H3N2))	ABG66981.1
(A/equine/Berlin/1/91(H3N8))	CAA11171.1
(A/duck/Hokkaido/238/2008(H9N2))	BAH23432.1
(A/duck/Mongolia/119/2008(H7N9))	BAH22785.1
(A/duck/Hokkaido/HY57/2005 (H9N4))	BAG69188.1
(A/duck/Mongolia/149/03(H10N5))	BAG66277.1
(A/duck/Hokkaido/95/1981(H8N4))	BAG66275.1
(A/duck/Miyagi/47/1977(H11N1))	BAG66272.1
(A/duck/Hokkaido/W87/2007 (H10N2))	BAG66264.1

Clustal Omega created the MSA and the phylogenetic tree. On analysis of the tree, it was seen that H3N2 and H3N8 were the most closely related strains while H10N5 and H8N4 were also closely related. H11N1 was the most distantly related strain to the remaining nine sequences.

Structural Analysis

From RCSB-PDB, the structure of M2 protein was obtained- PDBID- 5C02. The structure obtained was then visualized using RasMol. The number of H-bonds and di-sulphide bonds were calculated. M2 is a transmembrane protein with 97 amino acid residues present in Influenza virus. It comprises of 4TM helicases with N- terminal pointed outside and C- terminal pointed inside. This protein mainly functions as H+ channel. In an acidic environment, the pH sensor (His37) gets activated which in turn opens the "gate" (Trp41) and brings about selective permeability to H+ ions. This is a complex mechanism and it's achieved with the help of many other a.a units like asp44, val27 etc

Binding Site Prediction

The .pdb structure of M2 protein obtained from RCSB-PDB was uploaded to ConSurf Server in order to find the binding sites. Through the results obtained and from further literature search, we were able conclude that the binding sites for amantadine anti-viral blocker were Ser31 and Gly34

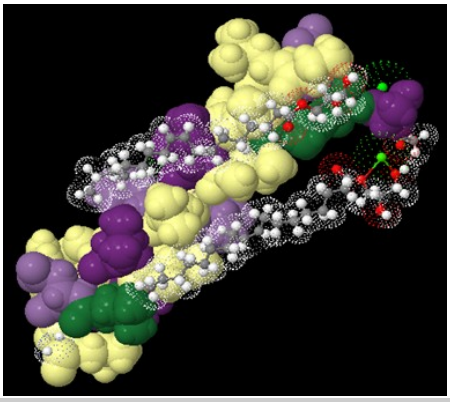

Fig 1: Binding Site Prediction using ConSurf Server

Protein Modelling and Docking

SPDBV and obtained the protein model of M2 protein which was then used as receptor and amantadine was used as ligand in Hex 6.3. Docking was performed and it was seen that there were 9 poses and the pose with the least energy had energy of -108.39 kJ

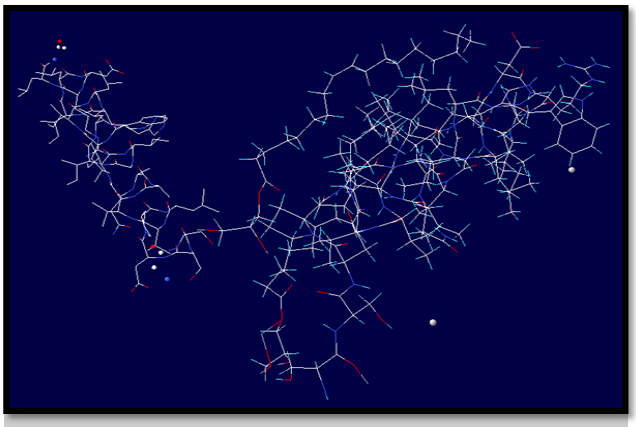

Fig 2: Protein Model obtained using SPDBV Version 4.1.0

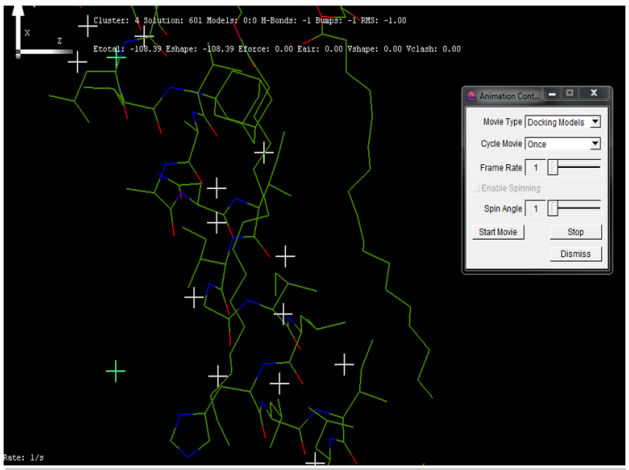

Fig 3: Docking in Hex 6.3 [E(total)= -108.39 kJ]

Visualization/Simulation

In order to understand the concepts better, we used two software- Blender for modelling and Unity 3D for creating simulations. All the necessary forces were considered and the simulation was created and was used to map the resistance potential on Influenza's H+ channel against the ant-viral blocker.

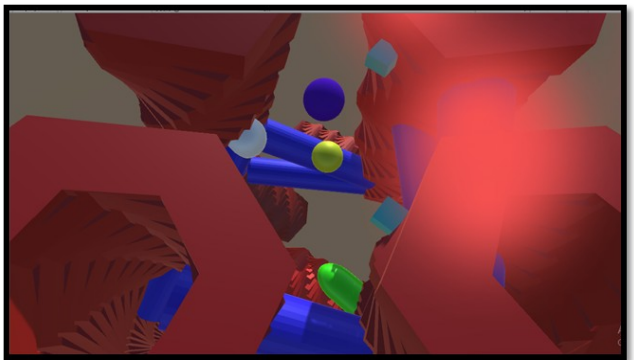

Fig 4: Simulations of H+ channel function using Unity 3D

Discussion

Through our efforts, we were able to map the resistance potential of Influenza's H+ channel against an anti-viral blocker (amantadine) and the phylogenetic analysis performed also enabled us to understand the evolutionary relationships between the viral strains. Through further literature search, it was understood that once the blocker attaches to the binding sites (Ser31 and Gly34), the channel gets inhibited (blocked), thereby hindering the movement of H+ ions from exterior to the interior of the virus. Results obtained from docking suggested that the pose with the least energy (-108.39 kJ) was the most stable interaction, thereby providing more solid evidence to support our efforts. .

An attempt was made to comprehend the concepts of drug delivery in a much more refined manner using advanced 3D simulation techniques. Mapping the resistance potential of such diseases can be used a platform to study and understand them in a better fashion. This information can be used to develop channel specific drugs in a more efficient manner.

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Conflict of interest

The author's declares none.

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