Higher Affinity Heptapeptides derivatives Against Influenza Hemagglutinin-Sialic Acid Identification for Curing Flu Virus Disease

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I. Introduction:

Influenza is a high mutability virus with many human-infecting strains. The virus has three types A, B, and C. each type has sup-types that are being differentiate by their surface proteins. The two main surface proteins are Hemagglutinin (HA) and Neuraminidase**.** There is 18 different HA and 9 different NA. based on a combination of one HA type and one NA type, a subtype is identified. For each of these subtypes, there are many strains. between April 12 2009 and April 10 2010, a strain of the virus (H1N1) infected around 60 million people in the U.S causing a pandemic, resulting in 12,469 casualties [1].

 One method the virus use to bind to human cells receptor is by binding of its HA to sialic acid. Sialic acid is a sugar attached protein units present on the surface on many cells on the upper respiratory track and erythrocytes. For the virus, it serves as an identifier for the viral host cell. Stopping this binding would be an excellent infection prevention strategy.

 Peptide therapy is a form of treatment that uses peptides as inhibitors for binding of viral agents with host cells. A few pentadecapeptides (a chain of 15 amino acids) was found to bind with HA mimicking the role of sialic acid [2]. On further study [3], those peptide chains were reduced in size to heptapeptides (a chain of seven a.a.) by docking simulation, protein-protein interactions prediction software based on crystal structure, that kept the pentadecapeptide affinity and ability to bind to HA. However, these heptapeptides were tested on selected strains of only few different subtypes (H1 & H3). However, those strains belong to virulent subtypes that infects humans that has been mutated from flu strains that infects either birds, pigs, or humans. In this study, we address the possibility of finding derivatives of these heptapeptides affinity by replacing amino acids (a.a.) with other a.a. with the same hydrophobicity and test them in a wider range of subtypes.

II. The Experiment:

1. Overview:

The heptapeptides chain id LVRPLAL (C1) has two essential a.a. for its binding to HA: Arginine (R3) and Proline4(P4) [2]. This chain was tested on two strains of the virus, A/Wyoming/03/2003(H3N2) and A/New Caledonia/20/99 (H1N1). We further take these peptides and test for their binding affinity to multiple strains of different subtypes. These strains were chosen for their host are human cells (Table.1).

|  |  |
| --- | --- |
| Subtype | strain |
| H1N1 | (A/California/07/2009) |
| H3N2 | (A/Brisbane/10/2007) |
| H5N1 | (A/Anhui/1/2005) |
| H7N9 | (A/Anhui/1/2013) |
| H5N1 | (A/turkey/Turkey/1/2005) |
| H1N1 | (A/Texas/05/2009) |

Table1. A total of 6 strains were randomly chosen, each from a different subtype.

B.    Methods:

**electronic prediction:** The main application of this step, is to reduce the time needed to test every hepta peptide one-point mutation.

This prediction will serve as time & cost saving pressures; to increase to affinity of heptapeptide chain we’re testing (LVRPLAL) we will substitute each a.a. acid with another

a.a. that has the same hydrophobicity. The exceptions are R#3 and P#4 since they have proven essential for the binding [3]. Even when we have these two constant a.a., we still have a very large number of possible chains (around 32768 different chain). This problem can be solved by using a prediction program called PRODOCK [4] that relies on the structure of HA to test for the affinity [5]. In brief, this program tests whether a peptide chain will bind to HA based on the crystal structure of that HA. The structure itself can be obtained from Protein Data Bank. for each successful binding the software will give a strength value. After comparing the results, we will test the 50 strongest chains. Narrowing the number of chains to 50 will reduce the cost of peptide synthesis down to about $2500

in total.

**wet lab execution**: the core method and the conformation step, a modified indirect enzyme-linked immunosorbent assay(iELISA).

Since we are testing short chain of a.a, it would be difficult to attach any chain with IgG without the possibility of losing it function. We can solve this issue by preparing our own wells using DLCM [6]. In brief, this method uses an in-well reaction in the following order: first, melaimide-activated bovine serum albumin (BSA-M) is dispended in microwell plates and incubated over night at 4 c. next, the plates will be extensively washed with distillated water. Next, the tested heptapeptide will be dissolved in melaimide conjugated buffer(HB). And a serial dilution with will be performed to measure the strength of the reaction after ELISA. Next, each diluted HB will be dispended on the pre-coated BSA-M plates. To allow cross-linking, we will incubate the plate for 2 hours at room. Finally, the plate will be washed with water.

After preparing the wells (fig.1, step1), we will add the selected strains of the virus (fig1, step2). After washing the wells to get rid of the unattached strains, we will add specific antibody against that strain (fig1, step 3). After washing the well, an HRP linked antibodies against the primary antibody (fig.1, step 4). After the final washing, H2O2(a substrate) & 2 2' azinobis (electron donor needed for the reaction), will be added to measure the strength of the binding giving a green color (fig.1, step 5). For each well’s column, a serial dilution will be performed. This will indicate how the specific affinity for that chain is. A comparison of rows will be performed. Each consecutive six rows have different strain but same mutated heptapeptide chain. The order of the chains is based on docking simulation.



Fig.1) testing for an influenza strain binding to a mutated heptapeptide using modified

indirect ELISA method(iELISA). Step 5 represents a successful binding detection.

Materials:

 Preparation of HRP-Antibody and it’s substrate is described in[7]. The different strains are sold by many companies like ATCC, the primary antibodies are sold by BioLegend, and the secondary are sold by Thermo Fisher. The primary antibody should be Rabbits and the secondary is goat’s anti-rabbit antibodies.

III. Discussion:

* One major issue we have with these heptapeptides that human testing is necessary to assure selective binding to HA. In addition, we suspect a dose failure without a proper method of delivery, the drug may be eliminated by the body faster than the timing needed for binding to the virus protein.
* This is a small test done on some random strains of the virus with limited resources. Overall, these heptapeptides are being tested for the affinity they had with HA. Further studies are recommended.
* One major goal we seek is providing some sort of first protocol against flu pandemic outbursts.
* In case of failure to produce a higher affinity chain to certain strain, we recommend preforming mutation with disregard to hydrophobicity.

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 DOI: [10.1371/journal.pone.0094392](https://dx.doi.org/10.1371/journal.pone.0094392)

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