Original Article

Nanobiological studies on drug design using molecular mechanic method

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Abstract

Background: Influenza H1N1 is very important worldwide and point mutations that occur in the virus gene are a threat for the World Health Organization (WHO) and druggists, since they could make this virus resistant to the existing antibiotics. Influenza epidemics cause severe respiratory illness in 30 to 50 million people and kill 250,000 to 500,000 people worldwide every year. Nowadays, drug design is not done through trial and error because of its cost and waste of time; therefore bioinformatics studies is essential for designing drugs.

Materials and Methods: This paper, infolds a study on binding site of Neuraminidase (NA) enzyme, (that is very important in drug design) in 310K temperature and different dielectrics, for the best drug design. Information of NA enzyme was extracted from Protein Data Bank (PDB) and National Center for Biotechnology Information (NCBI) websites. The new sequences of N1 were downloaded from the NCBI influenza virus sequence database. Drug binding sites were assimilated and homologized modeling using Argus lab 4.0, HyperChem 6.0 and Chem. D3 softwares. Their stability was assessed in different dielectrics and temperatures.

Result: Measurements of potential energy (Kcal/mol) of binding sites of NA in different dielectrics and 310K temperature revealed that at time step size = 0 pSec drug binding sites have maximum energy level and at time step size = 100 pSec have maximum stability and minimum energy.

Conclusions: Drug binding sites are more dependent on dielectric constants rather than on temperature and the optimum dielectric constant is 39/78.

Key Words: Binding site, influenza A (H1N1), molecular mechanic, neuraminidase enzyme

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INTRODUCTION

Many worldwide influenza epidemics have been recorded in the history of medicine; the first epidemic occurred in 1918 in Spain in which about 50-100 million people died:[1] Its reason was the H1N1 virus. The second epidemic occurred in 1957 in Asia, in which about 4 million people died and it was caused by the H2N2 virus. The third epidemic that killed about 1 million people occurred in 1968 in Hong Kong; the cause was the H3N3 virus. The most important matter about these viruses is their nature of being epidemic and pandemic.[2,3] In Iran in November of 2009 (Aban of 1388), about 2662 cases of influenza were proved using reverse transcription

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polymerase chain reaction (RT-PCR),[4] Nowadays this illness could be transmitted from human to human and it can transfer very quickly through sneezing, coughing and the secretions of the nose and mouth, and touching respiratory droplets.[5] This virus can stay alive for 2 to 8 hours, so it can transfer through objects, doors and public devices. It has been proven that the risk of transfer is higher in the first 4 hours after exposal.[6] We can recognize this illness by the use of Nasofarnex and cell culture and also RT-PCR; that would interpret good results[4] for special patients and groups (high risk) such as: adults, younger children, pregnant women, and diabetics. Influenza A virus belongs to the Orthomyxoviridae family and is classified as three types: A, B and C; Type A is clinically the most important one. The hosts of influenza A are human, swine, hen, horses and birds.[7-9]

The form of swine influenza virus is mostly spherical or ovoid [Figure 1] and sometimes long, spaghetti- like. They are composed of lipid membrane, glycoprotein molecules, HA and NA, and small amounts of trans-membrane protein, M2.[7-9] The genome of influenza A viruses consist of eight segments of single-stranded RNA of negative polarity, that code about 11-12 proteins.[7,8]

Proteins that are coded by Influenza A

Protein that is coded by segment 4, Hemagglutinin (HA), and by segment 6, Neuraminidase (NA), and the M2 protein are the most important proteins that employ important roles in drug design (Gong *et al*., 2007, and Bauer *et al*., 2009). There are double lipid layer subtype viruses Hemagglutinin (HA, 1-16) and Neuraminidase (NA, 1-9) on the membrane. Humans have Hemagglutinin H1 and H3 and Neuraminidase N1 and N2 (Gong *et al.)*.[1-8]

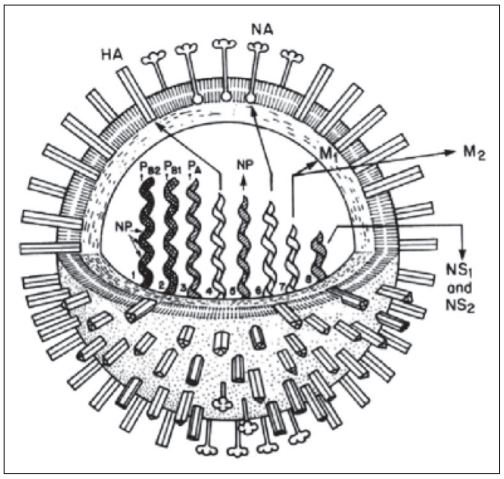

Figure 1: Schematic of the influenza A virus (Gong *et al*., 2007)

Neuraminidase

Crystallography and Neuraminidase (NA) enzyme were provided by Laver and his assistants in 1983 [Figure 2]. Neuraminidase (EC: 3.2.1.18) has an important role in the influenza virus and is the major and important goal for controlling the development of this illness. Spanish influenza that occurred in 1918 and its complex with Zanamivir (ZMR) was determined at 1.65-A and 1.45-A resolutions, respectively. The most important characteristic of this virus surface protein is its enzymic role. This protein consists of 4 subunits with asymmetric tetramer architecture which shape a β-sheet and the weight of each subunit is 200 KD.[10] Considering the sequence of amino acids, this enzyme has been conserved in active sites of different viral species. Babu and colleagues mentioned the conserved binding site theory in 2000. It must be noted that Ca ion enzyme has been observed near the conserved binding sites which its role is still not clear.[10] As has mentioned before, this enzyme has two chains of α and β.[10-14] Binding sites and active sites are conserved in different species [Figure 2]. The sequence of amino acids could be observed in Figure 2. Neuraminidase enzyme is responsible for decomposition or hydrolyzation of Sialic acid and the enzyme of virus enters the host's cell through this way.

Neuraminidase inhibitor

Zanamivir (Releza) and Oseltamivir (Tamiflu) are sensitive Neuraminidase enzyme inhibitors.[15-17] Amantadin and Rimantadin are reluctant inhibitors and the M2 protein (responsible for targeting the M2 protein-hydrogen pump channel), is an inhibitor that its consumption has been forbidden by Centers for Disease Control and Prevention (CDC).[11-4]

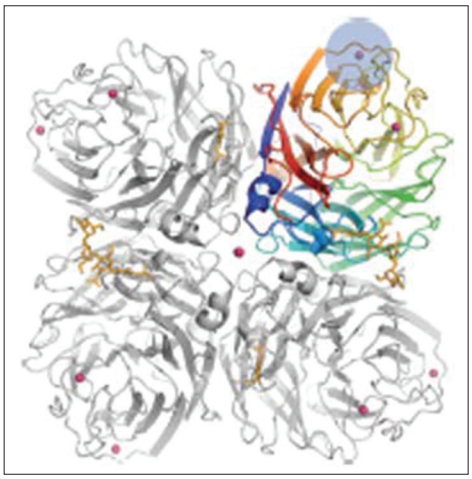

Figure 2: Crystal structure of the NA. The tetramer is composed of four identical monomers. The active site is located on top of the molecule (Xiaojin *et al*., 2008)

MATERIALS AND METHODS

In this study, data related to the classification of Neuraminidase (EC3.2.1.18), the weight of subtype 200KD,[10-18] and succession of amino acid in alpha and beta chains have been obtained from the Protein Data Bank (2010) and NCBI website. Then the whole structure of the enzyme was simulated using Argus Lab 4.0 software.

Since I PDB websites this protein is mentioned along with its inhibitor (Relenza = ZMR), during the simulation the inhibitor was simulated in connection to the active site, which in absence of the Sialic acid inhibitor the host's cell would be set in this site; on the other hand it is the inhibitor's binding site.[3] (Sialic acid's active site = Inhibitor's binding site)

There are 4 amino acids involved in altering or designing drugs that are: Asp151, Glu276, Arg152 and Arg371.[9-10] They are introduced as binding sites. These amino acids were stimulated with the drug (ZMR) in their own spatial situation. The obtained spectrums of NA protein proved that the amino acids had the most interactions with the drug in the 0active site. These amino acids have an important role in drug design that is the main goal of our study in WWthis research on NA binding sites.[19] Structural data about Neuraminidase enzyme's sequence, the quantity of amino acids, the quantity of water molecules in the proteins structure, for us,[20] the position of Ca ion, and drug's active sites and binding sites were obtained using Argus Lab software. There are 7009 atoms in this protein, and our study is on 4 amino acids and their inhibitors. In this study the behaviors of enzyme's binding site and active site was observed in aspect of potential energy levels in different dielectric constant and in 310 K temperature. The amino acids that are involved with the drug were studied in different organic solution such as Methanol, aqueous environment like water, and gaseous environmental like vacuum. Transferring the active site of enzyme to different phases in aspect of different dielectric constants was done using the HyperChem 6.0 software. Then the design of the organic environment was conducted through the Chem3D software and the most appropriate dimensions (X, Y, Z) for the box was calculated. The binding sites were also studied in different environments and in 310 K temperature (37DC, body temperature) at the step size of 100 psec.

RESULT AND DISCUSSION

After conducting the Molecular Mechanic study and gaining the potential energy by Monte Carlo method and studying the amino acids that were involved with the drug in different dielectric constant, the following results were concluded:

Binding site's behavior in vacuum

The Molecular Mechanic computations on the amino acids involved with the drug in vacuum displayed that in the primary stage of step size = 0 ps the account was in its highest level (90 kcal/mol), which decreased to energy level of 51 kcal/mol during time; in a 10 ps period of time, the range of decreasing energy could be observed clearly. The lowest level of energy was observed after 71-80 pSec [Figure 3a].

Binding site's behavior in water box

The Molecular Mechanic computations on the amino acids involved with the drug in aqueous environment

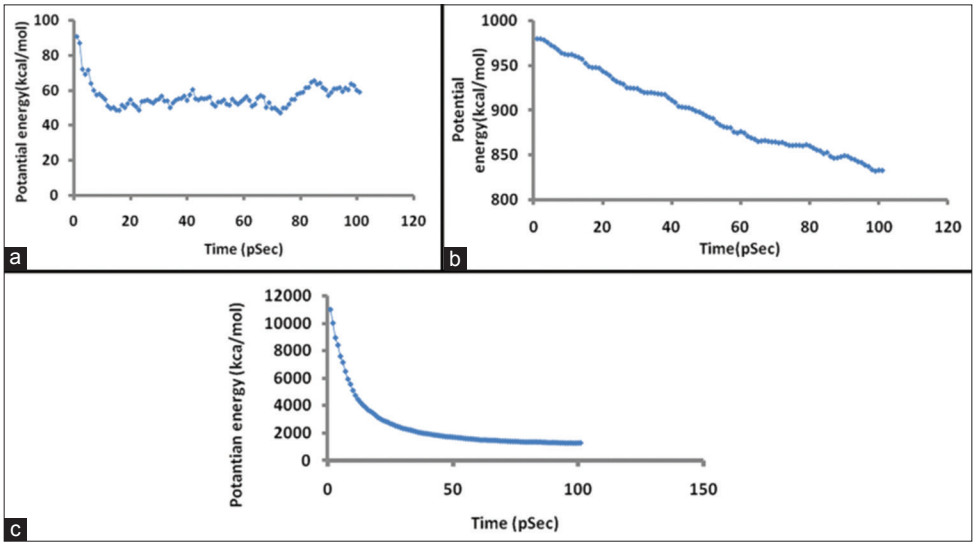

Figure 3: (a) Potential energy of binding site NA in vacuum. (b) Potential energy of binding site NA in Water box. (c) Potential energy of binding site NA in methanol

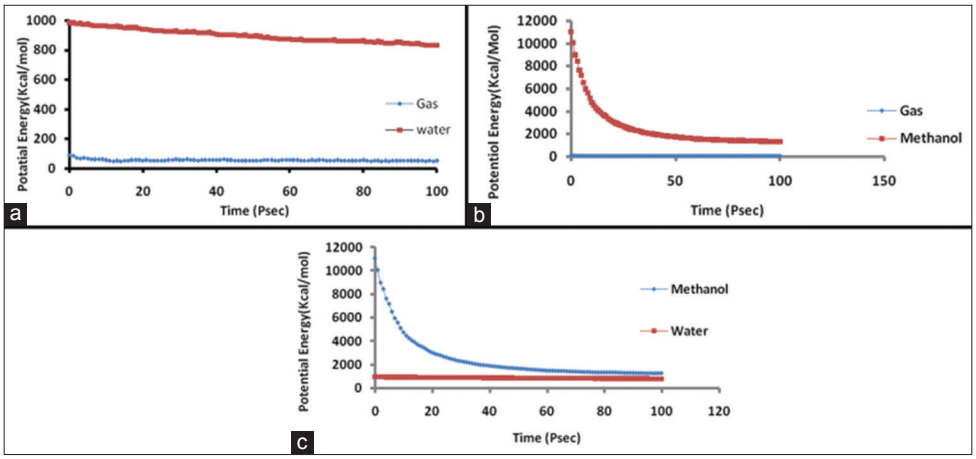

Figure 4: (a) Comparison of potential energy levels of binding site NA in water and vacuum. (b) Comparison of potential energy levels of binding site NA in methanol and vacuum. (c) Comparison of potential energy levels of binding site NA in water and methanol

showed that the level of the potential energy rose from 980 Kcal/mol to 833 Kcal/mol that can be seen in Figure 3b.

Binding sites behavior in Methanol box

The potential energy computations on the amino acids studied in a Methanol box revealed that the level of energy during step sizes 0 ps to 100 ps was decreased from 11034 Kcal/mol to 1310 kcal/mol [Table 1 and Figure 4]. The Monte Carlo chart also confirms that as time passes the level of energy would be decreased [Figure 3c].

Reports have shown that some drugs are synthesized in the organic environments and it is expected that some of these materials enter the body along with the drugs; therefore this is one of the reasons that the organic environment should be considered as the practical environment of the drug. Also the aqueos environment is the biologic situation of the human body in which protein's structure is hard and strong and it is the best biological solution for correct synthesizing and folding of protein structure.

On the other hand most of drugs need an aqueos environment for effectiveness. Also vacuum environment has basic importance for druggists. The dielectric constant is one of the most effective factors in determining the molecular structure and biological function. Besides statistical studies like Quantum Mechanic and Molecular Mechanic help to develop the drug design, but for good understanding of drug design we should have full and complete data about receptors, target sites and also binding sites.[21]

Table 1: Comparison of potential energy (Kcal/mol) at 310 K in different dielectrics

Time (pSec)	Water	Methanol	Vacuum
0	980.1198	11035.04	90.714
10	962.5878	4772.129	55.15993
15	949.2259	3714.928	51.88887
20	941.8292	3058.884	53.85016
25	929.5156	2649.583	51.81008
30	922.0881	2327.93	56.19719
35	918.8409	2088.523	53.13085
40	908.946	1938.632	58.25608
45	901.5344	1803.102	54.84956
50	892.2775	1709.7	52.80765
55	881.1722	1613.834	56.04681
60	874.9766	1521.245	56.43553
65	866.0952	1496.66	53.41846
70	863.9528	1447.458	54.52453
75	861.3597	1408.274	51.72659
80	858.2182	1379.762	55.73374
85	848.8647	1355.391	50.24827
90	848.8953	1329.733	53.4196
95	839.1277	1301.504	50.76776
100	833.1655	1310.315	51.2155

CONCLUSION

The study of binding sites showed that the system has the highest level of energy and the lowest stability in vacuum environment but during time the level of stability increases and the level of energy decreases, which is caused by the forces from inside the system and would make the amino acids and their aspects to change and find the best spatial conformity; in this situation binding sites have the highest stability, and it's the best condition for them. Vacuum environment has no logical effect on the stability of binding sites of Neuraminidase; therefore it has no effect on designing drug.[3]

The Molecular Mechanic computations (Monte Carlo) and comparison of the amino acids' stability are the main basics for designing drugs. On the other hand these 4 amino acids should find the best spatial conformity which means the highest stability level or

the lowest level of energy. So this study proves that water environment is better for Neuraminidase in comparison with organic and gaseous environments and in the stable biological condition, water part's dielectric constant is fit for biomolecules. Comparing the energy levels in natural temperature and fever temperature, it was revealed that when we face the Influenza enzyme it has no significant change in aspect of EPOT or Potential Energy and we can almost predict that stability or changes of enzyme behavior depends on dielectric constant, not the changes of temperature.

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