Structural Analyses of the Mode of Binding between T-Cell Surface Lycoprotein Cd4 with HIV gp120 C5 Protein Involved in HIV Infection and Search for Novel Phytocompounds for Its Treatment

Moupriya Nag[1], Dibyajit Lahiri[1], Soumalika Nath[1], Ritashree Deb[1], Asmita Das[1], Rina Rani Ray[2]

[1]Department of Biotechnology, University of Engineering & Management, Kolkata

[2]Associate Professor, Department of Biotechnology, Maulana Abul Kalam Azad University of Technology, Kolkata

Abstract :

The Human Immunodeficiency virus (HIV) belongs to subfamily retrovirus called lentivirus which results in the development of Acquired Immunodeficiency syndrome (AIDs)resulting in the destruction of the immune response system. This collapse may lead to the development of life threatening cancer and various types of opportunistic infections.In the first part, we intend to study the mechanisticdetailsofresidue specific binding and structural alterations in the T-cell receptor protein (PDB ID-1CDJ) after being attacked by the virulent protein (PDB ID-1MEQ) using standard bioinformatics tools.Tenofovir and efavirenz are known antiretroviral drugs for HIV with observed side effects. These drugs prevent early virus multiplication on host cells. Therefore, it is of interest to identify naturally occurring novel compounds to control viral growth. In the second part of this study, receptor protein was screened and their subsequent affinity towards the phytocompounds isolated from Neem (*Azadirachtaindica*) and Tulsi (*Ocimum sanctum* L.) plantswere studied using molecular docking tools. The study revealed that the natural phytocompounds have comparable activity to that of Tenofovir and efavirenz with high binding energy values with that of the target protein.Hence, it is of interest to consider these compounds for further *in vitro* and *in vivo* evaluation.

Keywords HIV, T-cell, Protein-protein interaction, ,Bioinformatics, Docking.

Introduction

The discovery of lymphotrophic retrovirus known as human immunodeficiency virus (HIV) dates in the year 1981 in the United States of America and since then it has become a major world epidemic affecting approximately 40 million people [1-3].

The mode of spread of the HIV occurs by progressive destruction of body's immune system thus making the body vulnerable to various infections and might also result in the development of cancer. These inflicted people are mostly prone to various types of opportunistic infections (OI) [4].The mode of spread mainly occurs through unprotected sex,contact with infected blood,contaminated needles,from affected mother to child through placenta and through the breast milk of mother to the child [5,6].

The retrovirus- HIV is enveloped with phospholipid when it buds out of the host cells at the time of replication. The extension from the envelope like structure is mainly composed of three to four gp41 glycoproteins that remains capped with gp 120 glycoprotein. The nucleocapsid present is HIV is composed of two strands of RNA. The virulence activity of HIV markedly increases due to the predominant existence of the enzymes reverse transcriptase, integrase and protease which remains integrated into the nucleocapsid region.

The helper T cell is the major target of the virus and thus our study focuses on the viral protein interaction with the T cell protein.

The penetration of the virus into the host system is predominantly dependent upon the mode of expression of the co-receptors. The glycoprotein gp 120 molecule of HIV binds with the CD4 cells upon the surface of the host cells. This is followed by the mechanism of binding of the second receptor glycoprotein with co-receptors like CXCR5 or CXCR4 that acts as a chemokine at the early stages of infection within the body. It is usually observed that CXCR5

is expressed in the later stage of the infections. The CXCR4 is mainly expressed in the major groups of the peripheral T cells and results in destruction as well as infection of the CD4 T-cells. [7-9].

The fusion of the viral envelope with that of the host cell membrane results in spread of infection.The reverse transcriptase enzyme present in the retrovirus like HIV allows the single stranded RNA to be generated into double stranded DNA.The integrase causes the integration of the viral DNA into the cellular chromosome. The replication of provirus occurs along with the host cellular chromosome during cell division.The integration of provirus into the host DNA provides the latency that enables the virus to evade host responses effectively.This allows the viral proteins to get assembled into the host cell protein making machinery.The viral protease enzyme allows the processing of translated polypeptide into the proteins which are then assembled into viral particles. The destruction of CD4+Tcells has attributed to both direct infection and Fas/Fas ligand mediated apoptosis [10]. HIV infected patients have shown considerable depletion of CD4+T cells within the gastrointestinal tract.

Thus, our aim in this study is to identify the changes in protein interaction of 1CDJ which is a Tcell surface glycoprotein made up of 178 amino acid residues with 1MEQ which is an exterior membrane glycoprotein of HIV during invasion and spread of infection. We also intend to study the molecular basis of the interactions between T cell proteins in presence of phytocompounds for its future therapeutic use in HIV pathogenesis. We built molecular models of the proteins and used the models to dock with phytochemicals [11-15].

The ûnal docked complexes were usedto compare the binding interactions between T cell protein and phytocompounds from plants. This analysis provides a rational framework to elucidate the complicated bimolecular mechanistic details of the interactions between these compounds and T cell protein [16-18].

Materials and Methods

Protein and ligand Search

UsingRCSB PDB[7], T cell receptorprotein(1CDJ) and gp120 C5 Domain of HIV viral protein (1MEQ) has beenidentiûed. The protein taken under consideration was 1MEQ and 1CDJ were found to have the best binding interactions.

Bioactive compounds from tulsi (apigenin) and neem (azadirachtin) were found from PubChem Database. The docking interaction was observed using the Auto Dock Tool [9]. *Preparation of protein structure*

The X-ray crystallographic structure of the protein from the Protein Data Bank was taken into consideration for this study. The ligand existing within the protein structure were removed from the binding site before it was docked using Auto Dock Tool.

Protein-Ligand and Protein-Protein Docking using Autodock4.2

Grid box generation

The grid file of the T cell receptor protein was generated using the Auto Dock Tool. The dimension of the grid box was such that it covered the entire structure of the protein and the ligand for studying the docking interaction. [19-21].

Molecular Docking of T-Cell Surface Lycoprotein Cd4 (1CDJ) with HIV gp 120 C5 Protein (1MEQ) using Autodock4.2

Protein-protein docking of T-Cell Surface Lycoprotein Cd4 (1CDJ) with HIV gp 120 C5 Protein (1MEQ) has been done usingAutodock.Thesameprotocolwas followed in presence of phytochemical extracts of Neem and Tulsi [19].

Ligand docking

The docking interaction was measured using AutoDock4.2 software. [11]. This study was performed to observe the possible mode of interaction between the ligand and the receptor protein in all possible orientations. The interaction study was based on Lamarckian Genetic Algorith (LGA) method. The conformation possessing the lowest value of energy was chosen to have the best docking interactions. [20,21].

Drug scans

To determine the drug likeness propertiesof the ligand the Lipinski's rule was tested [22]. The study also encompasses on the quantity of hydrogen bond acceptor, the amount of hydrogen bond donors, Log P, and the molecular mass of the drugs. In addition, the admetSAR calculated various attributes of the drugs like Blood brain barrier (BBB) penetration, Human Intestinal absorption, Caco-2 permeable, Aqueous solubility, P-gp substrate and inhibitor, CYP450 substrate and inhibitor, CYP IP, ROCT, HERG inhibition, and toxicity parameters. The ligand in SMILE format was uploaded to the analysis software websitewhere Lipinski Filters were used. The same applies to Molinspiration and admetSAR websites.

Results

Docking with Autodock4.2

Docking between 1CDJ and 1MEQ

Molecular docking enables a scientist to virtual screen a number of candidate compounds based on their binding orientation and binding ability with a target particle. It also allows one to select compounds with strong affinity for the target site. In the present work, binding interaction between T cell receptor protein (1CDJ) and gp120 C5 Domain of HIV viral protein (1MEQ) is evaluated. Fig. 1 and Table 1 depicts the best docked structure based on highest binding energies. The residues taking part in active interaction is depicted in Table 2.

Docking between 1CDJ and phytocompounds from Neem and Tulsi

After successful identification of the binding sites in the receptor protein, phytocompounds of tulsi and neem were docked in silico with the receptor proteins and docking sites were compared with viral proteins for its subsequent use as putative drugs. The docked ligand molecules were selected based on highest binding energy and good interaction with the active site residues and the results are shown in Table 2 and Fig 2.

Drug Scan (ADMET Analysis)

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME/Tox) are main five parameters to test the drug likeness of a molecule. Based on the docking study, two potential ligands molecular structure was submitted to Molinspiration and admetSAR servers to determine their different ADMET properties. Apigenin (from tulsi) and Azadirachtin (from neem) followed the Lipinski's rule of five without any violations with respect to an octanol-water partition coefficient (LogP d" 5), molecular weight (d" 500 KDa), number of H-bond donors (d" 5), number of H-bond acceptors (d" 10), molecular refractivity (40–130) as tabulatedinTable 3. Thus, Apigenin and Azadirachtin was considered as a potential drug candidate for further ADMET analysis.

In ADME assessment, different pharmacokinetic and pharmacodynamic parameters were considered such as aqueous solubility, human intestinal absorption, blood–brain barrier penetration, Caco-2 permeability, cytochrome P450 inhibition, renal organic cation transportation, HERG inhibition. The results have been summarized in Table 4. Moreover, the analysis performed on admet SAR revealed that both Apigenin and Azadirachtin had no substantial ADME properties that could cause adverse effects in humans.

Discussions

The study revealed that natural products interacted quite efficiently with that of the target protein. Azadirachtin is a limonoid class that is most active secondary metabolite present in neem seeds. Neem plant is well known for its medical properties. Neem plant has shown its antiviral activity due to the presence of Azadirachtin. Considering this, Azadirachtin can be used as an antiviral drug for HIV[22,23].

Conclusion

Molecular docking of Azadirachtin with T-cell proteins shows stronger bindingenergy having good ADMET property compared to Apigenin. Therefore, it is of importance to pursue Azadirachtin as a molecule of interest in this context for further in vitro and in vivo studies.

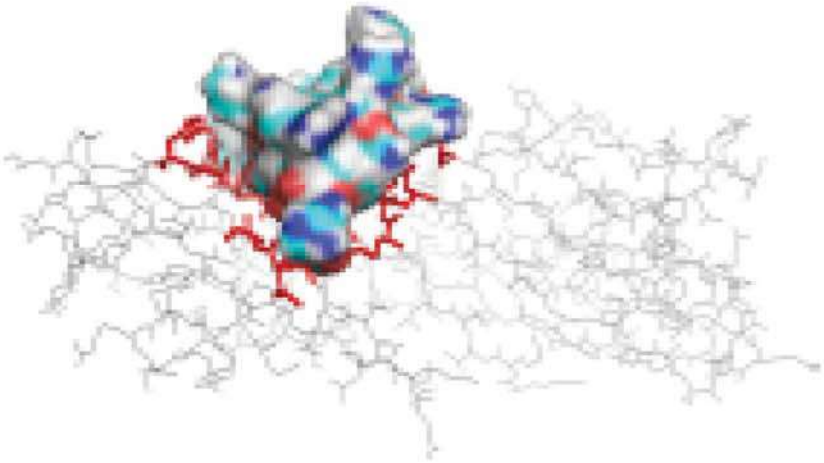

Fig. 1 Lowest energy docked poses of HIV protein (1CDJ, coloured surface image) at the binding site of T cell receptor protein (1MEQ, gray sticks in colour). Nearby interacting residues of receptor molecules are shown in red colour sticks.

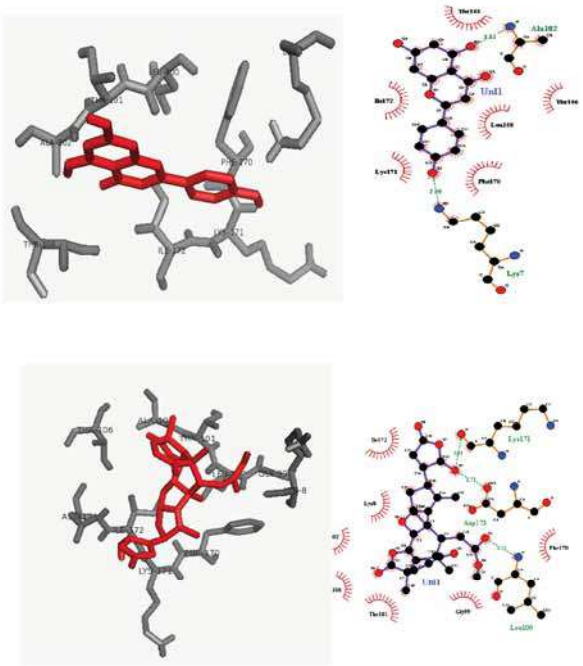

Fig 2.Molecular docking interaction between T cell receptor molecule with A) Apigenin and B) Azadirachtin. Binding energy and their ligplot interaction energy were calculated based on hydrogen bonds, polar, cation-pi, hydrophobic and other energies. Azadirachtin showed the highest binding energy with receptor protein as compared to the apigenin based on the molecular docking and ligplot interaction.

Table 1 Azadirachtin showed the highest binding energies (Kcal/mol) with comparison to Apigenin while docking with Receptor proteins 1CDJ

Ligands	**T cell receptor protein (1CDJ)**
HIV viral protein 1MEQ	-4.86 Kcal/mol
Apigenin	-4.62 Kcal/mol
Azadirachtin	-6.17 Kcal/mol

Table 2 Residual numbers of amino acids, involved in PPIs, for virulent and ligand docked complex with T cell receptor protein, are listed

Ligand	**Docked complex**	**Binding site residues' number of T-cell receptor protein**
1) HIV viral protein	K2, E21,R23, V17, P5, K12, K22	N73, K171, K7, I172, N103, E119, D10, A102, T101, H107, K171, L100
2) Apegenin		T101, A102, T106, L100,F170, K7, K171, I172
3) Azadirachtin		K171, D173, F170, L100, G99, T101, T106, K8, A102, I172

Table 3: Molinspiratin web server was used to calculate Lipinski's rule of five drug-likeness properties of potential compounds. Apigenin and Azadirachtin didn't violated Lipinski's rule of five for druglikeness properties.

Plant	**Ligands**	**LogP(<5)**	**TPSA (<140 Å)**	**MW (<500)**	**nOH (H donor< 5)**	**nOHNH (H acceptor <10)**	**Volume**	**n Violation**	**Molar refractivity (40-130)**
Tulsi	Apigenin	2.46	90.89	270.24	5	3	224.05	0	118.725174
Neem	Azadirachtin	1.29	125.45	498.53	9	1	433.44	0	--

Table 4: ADMET properties of Apigenin&Azadirachtin predicted from admetSAR server. Azadirachtin showed better BBB, HIA and Caco-2 permeability as compared to Tenofovir and Efavirenz. Whereas the remaining parameters, Azadirachtin showed almost similar to Tenofovir and Efavirenz

ADMET	Apigenin	Azadirachtin	Tenofovir	Efavirenz
Blood-Brain Barrier BBB	+	+	+	+
Human Intestinal Absorption (HIA)	+	+	+	+
Caco-2 Permeability	+	+	+	+
Aqueous solubility	-2.77	-4.45	-2.96	-4.52
P-glycoprotein Substrate	-	+	+	-
P-glycoprotein Inhibitor	-	+	-	-
CYP450 2C9	-	-	-	-
CYP450 2D6	-	-	-	-
CYP450 3A4	-	+	-	+
CYP450 1A2	+	-	-	+
CYP450 2C9	+	-	-	-
CYP450 2D6	-	-	-	-
CYP450 2C19	+	-	-	-
CYP450 3A4	+	-	-	-
ROCT	-	-	-	-
HERG-I	Weak	Weak	Weak	Weak
HERG-II	-	-	-	-
Ames Toxicity	-	-	-	-
Negative for genotoxic carcinogenity	Yes	Yes	Yes	Yes
Negative for nongenotoxiccarcinogenity	Yes	Yes	Yes	Yes

Abbreviations: ADMET, absorption, distribution, metabolism, and excretion-toxicity; BBB, blood–brain barrier penetration; HIA, human intestinal absorption; Caco-2, Caco-2 permeability; CYP, cytochrome P; ROCT, renal organic cation transportation; HERG, human ether-a-go-go-related genes inhibition; P-gp, permeability glycoprotein; +, present; -, not present.

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