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RESEARCHARTICLE

STRUCTURAL ANALYSIS OF HIV-2 REVERSE TRANSCRIPTASE AND ITS DOCKING WITH PHYTOCHEMICALS

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ABSTRACT

Human Immunodeficiency Virus (HIV) is a rapidly expanding global pandemic. Despite more than a decade of intense research to understand the HIV pathogenesis aimed at developing an effective therapy for AIDS, achieving a true eradication of HIV remains a daunting challenge. However, significant progress has been made in the management of HIV-1 replication using potent inhibitors. HIV-2 serotype of HIV was determined to be a cause of disease in the parts of the West African population, and there is evidence for its spread to Europe and Asia. It has also been found that HIV-2 reverse transcriptase (RT) demonstrates intrinsic resistance to non-nucleoside RT inhibitors, one of the two classes of anti- AIDS drugs that target the viral RT. The given article is an attempt towards designing a potent drug that can block the activity of HIV-2 RT. This has been achieved by conducting a comparative analysis between the reverse transcriptase from the two strains of HIV viz. HIV-1 and HIV-2. After the comparative analysis of two genomes and structure of the enzyme, molecular docking of the HIV-2 RT was performed using phytochemicals in order to gain insights pertaining to mechanism of binding of the ligand to the receptor. Docking results revealed that Curcumin, Astralgin and Tiliroside bind to pocket 2 (Pkt 2) with higher binding energies as compared to pocket1 (Pkt1).

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a medical condition that results in weakening of the natural defense machinery, the immune system of the individual resulting in its susceptibility to a plethora of opportunistic infections and tumors to invade the body. This susceptibility gets worse as the disease continues (Sepkowitz 2001). Since the first cases of AIDS were identified in 1981, more than 30 million people have died from AIDS (as of 2013). An estimated 1.8 million people died as a result of AIDS in 2010 alone. AID Siscaused by Human Immunode ficiency Virus (HIV) belong ingto the genus *lentivirus* (Weiss 1993).The genus Lentivirus, itself is a subset of the Retroviridae family of RNA viruses that includes viruses which share acommon replicative cycle. Infection caused by each of the lentiviruses including HIV-1andHIV-2,Simian ImmunodeficiencyVirus(SIV) cause cellular and systemic events that initiatediseaseprocess.It is already reported that Human Immunodeficiency Virus (HIV) enters human body through the transfer of blood, semen, vaginal fluid or breastmilk through four major routes of transmission that includes unsafe sex, contaminated needles, breast milk, and from mother to fetus. Once HIV enters the human body, itsprimary target is the immune cells of the body i.e. T-helper cells (specifically, CD4+ T cells), macrophages, and dendritic cells (Cunningham *et al.,*2010). HIV infection leads to low levels of CD4+ T-cells through three main mechanisms: First, direct viral killing of infected cells; second, increased rates of apoptosis in infected cells; and third, killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. Reverse transcriptase converts the single stranded HIV RNA genome to double stranded DNA copy by catalyzing both DNA-dependent, RNA-dependent polymerization as well as RNase H cleavage activity to remove the RNA template after the synthesis of its DNA. Due to these unique catalytic properties, Reverse transcriptase (RT) has been the target enzyme for many antiviral therapeutic agents used in treatment of AIDS, including nucleoside and non-nucleoside analogues (De Clercq 1994). Reverse transcriptase inhibitors (RTIs) are a class of antiretroviral drug used to treat HIV infection by inhibiting the ability of the enzyme to form DNA from RNA. RTIs come from three main types: First, Nucleoside Analog Reverse- Transcriptase Inhibitors (NARTIs

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or NRTIs); second, Nucleotide Analog Reverse-Transcriptase Inhibitors (NtARTIs or NtRTIs). The mode of action of NRTIs and NtRTIs is essentially the same; they are analogues of the naturally occurring deoxynucleotideneeded to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain (Paul*et al.,* 2006). Third, Non-Nucleoside Reverse-Transcriptase (NNRTIs), on the other hand, blocks reverse transcriptase by binding at a different site on the enzyme. NNRTIs are not incorporated into the viral DNA but instead inhibit the movement of protein domains of reverse transcriptase that areneeded to carry out the process of DNA synthesis. NNRTIs are therefore classified as non-competitive inhibitors of reverse transcriptase.The crystal structure of HIV-1 reverse transcriptase consists of two subunits p66 and p51 forming a heterodimer (Rodgers*et al., 1995*). The larger p66 subunit contains the fingers, palm, and connection subdomain as well as the RNaseH domain. The p51 is a product of the same gene as the p66 subunit, however the RNaseH domain is absent as a result of the proteolytic cleavage. HIV-2 RT on the other hand forms a more stable p68/p55 heterodimer compared with p66/p51 HIV-1 RT heterodimer (Ren *et al.,* 2002). Although the two molecules possess high degree of sequence similarities, the difference lies in the kinetic parameters for RNaseH and the polymerase. In case of HIV-1 RT, the RNA/DNA binding pocket is blocked by thumb of p51 whereas it is open in case of HIV-2 RT.Moleculardockingisamethodwhichpredictsthe preferredorientationofonemoleculetoa secondwhen bound toeachothertoformastable complex.Theaimofmoleculardockingisto evaluatethefeasiblebindinggeometriesofaputativeligandwithatar getwhosetargetsiteis known.Thesebindinggeometriesareknownasbindingposesthatin cludesboththepositionof ligandrelativetothereceptorandconformationalstateofthe ligandandthereceptor.The dockingprocedureconsistsof(i)characterizationofbindingsite;(ii) positioningofligandtothe bindingsite;and(iii)evaluatingthestrengthofinteractionforaspecif icligandreceptorcomplex. Theligandsusedtodocktheenzymewerefivephytochemicalsthatha vealreadybeenprovedto have anti-HIV activity

MATERIALS AND METHODS

Selection of binding pockets

The three dimensional structure of HIV-2 reverse transcriptase was retrieved from Protein Data Bank (PDB-id: 1MU2). The structure was then searched for possible binding pockets using LIGSITEcsc server (Hendlich *et al.,* 1997).The server predicted two possible binding pockets which were named as Pkt-1 and Pkt-2 respectively.

Ligand selection

Five phytochemicals with a known anti-HIV activity such as lycopene, curcumin, astralgin, tiliroside and 1-deoxynojirimycin were selected (Abhik Seal *et al.,* 2011). The 2D structures of these phytochemicals were retrieved from PubChemdatabase (http://pubchem.ncbi.nlm.nih.gov/). These 2D structures were then converted to 3D structures using openbabel (Figure1). These structures were evaluated for their drug likeliness based on Lipinski's Rule of five. However, later on, lycopene molecule was excluded from the docking studies as it did not obey Lipinski's Rule of five.

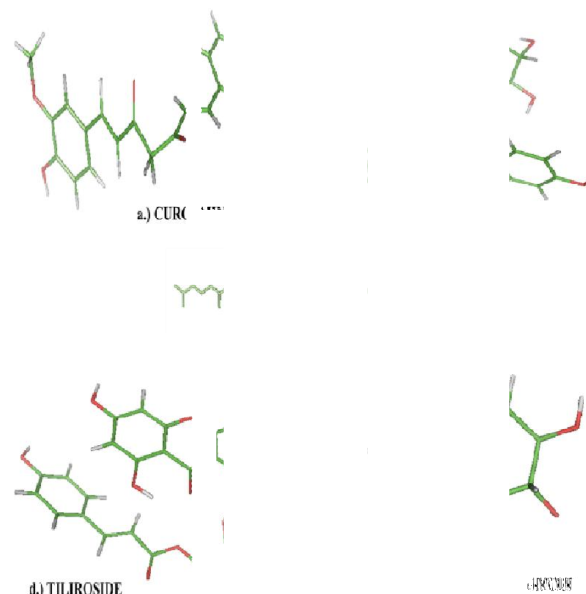

Figure 1.Structuresofthephytochemicalsusedasligandsfordockingwithreceptor (PDB id: 1MU2): a) Curcumin; b) Astralgin; c) Lycopene; d) Tiliroside; e) 1-Deoxynojirimycin

Protein-Ligand docking

The docking of Phytochemicals (ligand molecules) with HIV-2 RT (receptor molecule) was then performed using Autodock 4.0 and Argus Lab (Thomson 2004).

Docking using Argus Lab

The coordinates of PDB id: 1MU2 were provided as an input for the Argus Lab and the residues of the binding pocket were selected as the possible binding site. Then, the ligand file was uploaded. After calculation of grid parameters, the two structures viz. ligand and receptor were docked. The results obtained were ranked by energy values and their binding poses. The best possible conformation was saved as a PDB interaction analysis.

Docking using Autodock 4.0

Docking was performed with both the binding pockets viz. Pkt 1 and Pkt 2. Prior to loading the molecule, all heteroatoms were removed and hydrogen atoms were added to the enzyme to generate a PDBQT file. Then ligand PDB files (curcumin.pdb, astralgin.pdb, tiliroside.pdb, 1-deoxynojirimycin.pdb) were added each time to select bonds about which segments of the ligand will be rotated. Various docking parameters like torsion angles in the ligand were calculated and grid parameters were set to define the region for

docking the ligand resulting in the generation of GPF file. Autodock runs in the directory where the macromolecule, ligand, grid parameter file (GPF), docking parameter file (DPF) and maps are located. Upon completion of the program DLG files for several docked structures were obtained. The resulting files were then analyzed using Pymol (DeLano2008) to find possible hydrogen bonds present between the ligand and the interacting residues.

Table1.Interaction energiesobtained using the dockingofdifferentphytochemicalswithtwo diferentpockets. Column2 provides the results of dockingwith ArgusLAband Autodock 4.0with phytochemicalsinPkt1 and column 3 gives the interaction energies ofphytochemicalswithPkt2 respectively

Ligands	Pkt1	Pkt2
CURCUMIN		
1. Bindingenergy	-3.11kcal/mol (Autodock) -9.189kcal/mol (ArgusLab)	-5.11kcal/mol (Autodock) -9.2423kcal/mol (ArgusLab)
2. Interactingresidues	Tyr183,Met184	His360, Arg365, Asn403,Tyr404,Trp405, Gln406Gly503 16.927,12.131, 11.355
3. Grid parameters	17.381,10,10	
ASTRALGIN		
1. Bindingenergy	-2.19kcal/mol (Autodock) -7.158kcal/mol(ArgusLab)	-4.44kcal/mol (Autodock)-8.267kcal/mol(ArgusLab)
2. Interaction residues	Tyr183,Met184,	His360, Tyr404, Trp405, Gly503,Ala506, Ser507
3. Grid parameters	19.027,11.495, 13.247	12.082,11.420, 10
TILIROSIDE		
1. Bindingenergy	-2.19kcal/mol (Autodock) -8.577kcal/mol(ArgusLab)	-3.73kcal/mol (Autodock) -8.176kcal/mol(ArgusLab)
2. Interaction residues	Glu89, Gln91, Leu92,	His360, Arg365, Tyr404, Trp405, Gly503,Ala506, Ser507
3. Grid parameters	14.311,14.081, 19,909	10,12.154,15.464
1-DEOXYNOJIRIMYCIN		
1. Bindingenergy	-2.29kcal/mol (Autodock) -6.141kcal/mol(ArgusLab)	-1.87kcal/mol (Autodock) -6.580kcal/mol(ArgusLab)
2. Interactingresidues	Arg172, Asn175, Ile180	Trp405, Gly503, Ser507
3. Grid parameters	10,10, 10	10,12.082,11.420

RESULTS AND DISCUSSION

Using the Pocketfinder server, two pockets namely, Pkt1 and Pkt2 were identified and docking study using the phytochemicals was performed using the identified pockets.Out of the two identified pockets, the residues present in Pkt2 showed better binding affinity as compared to the residues in Pkt1 with phytochemicals. The structural and interactionanalysis revealed the presence of hydrogen bonding with the residues His360, Arg365, Tyr404, and Trp405 present in Pkt2 with all the phytochemicals under study. The detailed analysis of the atoms that are involved in H-bonding of residues in receptor with that of ligands is shown (Figure 2 and Figure 3).

It was also observed that the phytochemicals viz. Curcumin,

Astralgin, and Tiliroside were interacting with Pkt2 with more number of H-bonds as compared to Pkt1.

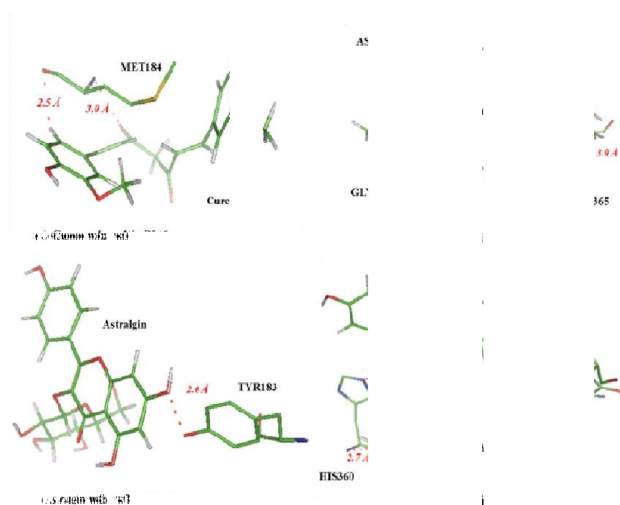

Figure 2. Interaction between the amino acid residues

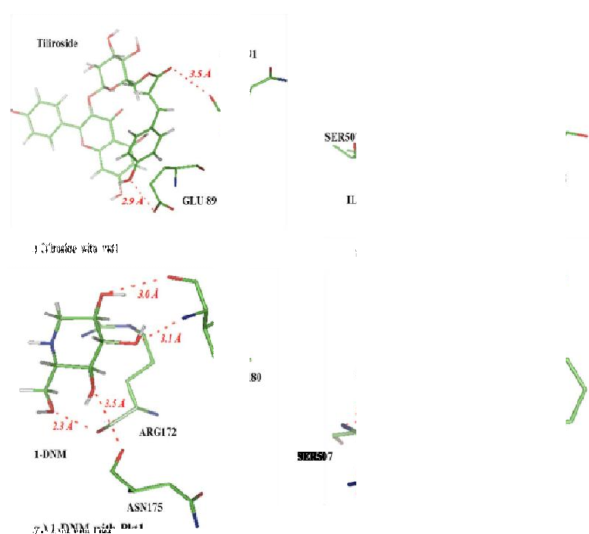

Figure 3.Interaction between the amino acid residues of Pkt1 and Pkt2 with the phytochemicals viz. Tiliroside and 1-DNM.

Conclusion

The values of interaction energies of all the phytochemicals that were used as ligand molecules to dock with the receptor molecule were given in Table 1.Among all the phytochemicals under study, curcumin was found to bind with both the pockets of the receptor molecule with maximum interaction energy. (Table 1) The values of interaction energies obtained due to the binding of Curcumin with receptor was found to be maximum employing both the rigid and flexible docking methods. The rigid docking was performed using ArgusLab, and flexible using was performed using Autodock 4.0. The molecular drawings were prepared using Pymol. The interaction analysis also revealed the presence of maximum number of H-bonds in case of curcumin interacting with residues of Pkt2. Docking of curcumin to Pkt2 shows best results with the highest binding energy of -9.24 kcal/mol using ArgusLab and -5.11kcal/mol using Autodock followed by astralgin and tiliroside. However, the H-bonding pattern in case ofcurcumin, astralgin and tiliroside shows on an average five hydrogen bonds between the ligand and the binding pocket. The detailed information pertaining to possible H- bonds is listed in Table 2 of SI.

Conclusion

In the present study it has been concluded that despite of high amount of sequence similarities between the two enzymes, certain mutations in the amino acids result in different structure and altered activity towards most of the common inhibitors, making it a possible element of research. These variations call for the search of several different drug targets that can block the reverse transcriptase activity of the enzyme thereby restricting the viral replication inside the host cell. Phytochemicals offer a better alternative to chemically synthesizedmoleculesasligands.Thisis becauseoftheir increased biocompatibilityandlesserchancesoftoxicity. Thedockingresults revealedthebindingofcurcumin, astralginandtilirosidetoPkt2withhigherenergies.This suggeststhat Pkt2 servesas agood bindingsitetothesephytochemicals.

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SupplementaryInformation

Table 1.Phytochemicals thatwereselected based onLipinski's Ruleof Five

	Lycopene	Curcumin	Astralgin	Tiliroside	1-DNM
CID	446925	969516	5282102	5320686	44387838
Average Molecular weight (g/mol)	536.87	368.3799	448.37	594.5	163.172
LogP	15.6	3.2	0.7	2.5	2.3
Donor	0	2	7	7	5
Acceptor	0	6	11	13	5

Table2.Possible Hydrogen bonds between theresidues inreceptormolecule with the Phytochemicals and their respectivebond lengths

Curcumin-Pkt1

O(MET184)---------------------------H(DEF)3.0Å
N(MET184)---------------------------O(DEF)2.5Å

Curcumin-Pkt2

NE(ARG365)-------------------------O(DEF) 3.0Å
NH2(ARG365)-----------------------O(DEF)2.3Å
O(ASN403)---------------------------O(DEF)2.0Å
OE1(GLN406)------------------------O(DEF) 3.4Å
O(GLY503)---------------------------O(DEF)2.6Å

Astralgin-Pkt1

OH(TYR183)-------------------------O(DEF)2.0Å

Astralgin-Pk2

NE2(HIS360)-------------------------O(DEF)3.0Å
N(HIS360)----------------------------O(DEF)2.7Å
O(HIS360)----------------------------O(DEF)2.6Å
OH(TYR404)------------------------O(DEF)3.2Å
NE1(TRP405)-----------------------O(DEF)2.9Å
OG(SER507)-------------------------O(DEF)3.4Å

Tiliroside-Pkt1

O(GLN91)----------------------------O(DEF)3.5Å
OE2(GLU89)-------------------------O(DEF)2.9Å

Tiliroside-Pkt2

NH1(ARG365)-----------------------O(DEF)3.1Å
NH2(ARG365)---------------------- O(DEF)3.3Å
OH(TYR404)-------------------------O(DEF)3.4Å
N(GLY503)------------------------- O(DEF)2.9Å
N(ILE504)--------------------------- O(DEF)2.7Å
N(SER507)---------------------------O(DEF)3.3Å

DNM-Pkt1

O(ARG172)---------------------------O(DEF)2.1Å O(ASN175)---------------------------O(DEF)3.1Å
N(ILE180)---------------------------O(DEF)3.1Å O(ILE180)-----------------------------O(DEF)2.1Å

DNM-Pkt2

N(SER507)----------------------------N(DEF) 2.9Å
O(GLY503) --------------------------N(DEF) 2.7Å
NE1(TRP405) ------------------------ O(DEF)3.1Å

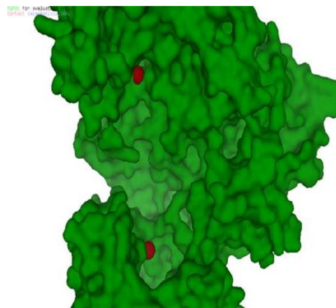

Figure 1.Possible bindingpockets(Pkt1 andPkt2showninred)aspredictedbyLIGSITEcsc.