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Full Length Research Paper

Possible role of 2, 2'- (Diazinodimethylidyne) di - (o-phenylene) dibenzoate, a novel hydrazine as an anti – HIV agent

Rita Ghosh*, Dipanjan Guha, Sudipta Bhowmik and Angshuman Bagchi

Department of Biochemistry and Biophysics, University of Kalyani, Kalyani –741235, W. B., India.

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A number of anti-HIV agents act by inhibiting specific steps in the lifecycle of HIV. Inhibition of reverse-transcriptase (RT), a multi-functional enzyme is an important option. The interaction of 2, 2´-(Diazinodimethylidyne)-di-(o-phenylene)-dibenzoate (HZ) with HIV1-RT was studied to explore its possible use in anti-HIV therapy. This novel hydrazine derivative undergoes strong interaction with a number of amino acid residues present in its catalytic domain, some of which are catalytically important like Asp-110, Asp-185 and Asp-186: significant among them is the Asp-186 residue that is highly conserved. Our studies may be useful in the field of structure based drug development in anti HIV therapy.

Key words: HIV 1, drug development, NNRTI, Hydrazine derivative, molecular modeling.

INTRODUCTION

HIV like all retroviruses requires for its infection the integration of double- stranded viral DNA into its host genome. HIV contains two identical single stranded viral RNA molecules (Takahashi et al., 2002). The reverse transcriptase of human immunodeficiency virus (HIV-RT) is responsible for the conversion of the HIV single stranded RNA genome into double stranded DNA (Basu et al., 2008; McBurney et al., 2006). This multi functional enzyme has RNA-dependent DNA polymerase activity, DNA-dependent DNA polymerase activity, strand displacement, strand transfer and RNase H activities (Lanciault and James, 2004). HIV-RT is a heterodimer composed of two subunits, p66 and p51 having molecular weights 66 and 51 kDa, respectively (Lightfoote et al., 1986; Jacobo-Molina and Arnold, 1991).

The polymerase domain is localized at the N- terminus of p66 and the 15 kDa C-terminal domain of p66 contains the RNase H active site (Szilvay et al., 1993). HIV RNase H is a 130 amino acid domain of the viral reverse transcriptase and is indispensable for the replication of genomic RNA to double stranded DNA. RNase H activity is to degrade the RNA from the RNA-DNA duplex (Tanese et al., 1991), so that the newly formed DNA can act as the template for the DNA dependent DNA polymerase activity in order to form the double stranded viral DNA and the virus can start its lifecycle inside the host cell (Dudding et al., 1991). Biochemical as well as structural analyses show the spatial distance between the two active sites (the polymerase active site and RNase H active site) was about 18 - 19 nucleotides in length when RT is bound to a duplex substrate (Furfine and Reardon, 1991; DeStefano et al., 1991; Gopalakrishnan, 1992; Jacobo-Molina et al., 1993). An effective therapeutic approach against HIV infection is to inhibit the RT activity.

There are two types of inhibitors - the nucleoside/nucleotide RT inhibitors (both called NRTIs for simplicity) and the non nucleoside/nucleotide RT inhibitors (NNRTI) that can act as anti-RT drugs. Some NRTI, 3'-azido-3'-deoxythymidine (AZT, zidovudine) (Fischl et al., 1987), 2',3'dideoxycytidine (ddC, zalcitabine) (Yarchoan et al., 1988), 2',3'- dideoxyinosine (ddI, didanosine) (Lambert et al., 1990; Cooley et al., 1990), 2',3'-didehydro-3'-deoxy-thymidine (D4T, stavudine) (Riddler et al., 1995) etc. are nucleoside prodrugs that are converted by cellular enzymes to the active deoxynucleotide triphosphates that lack a free 3'- hydroxyl group and lead to a chain

*Corresponding author. E-mail: rghosh_bcbp@klyuniv.ac.in, ritadg2001@yahoo.co.in.

Abbreviations: NRTIs, Nucleoside/nucleotide RT inhibitors; **NNRTI,** Non Nucleoside/nucleotide RT inhibitors.

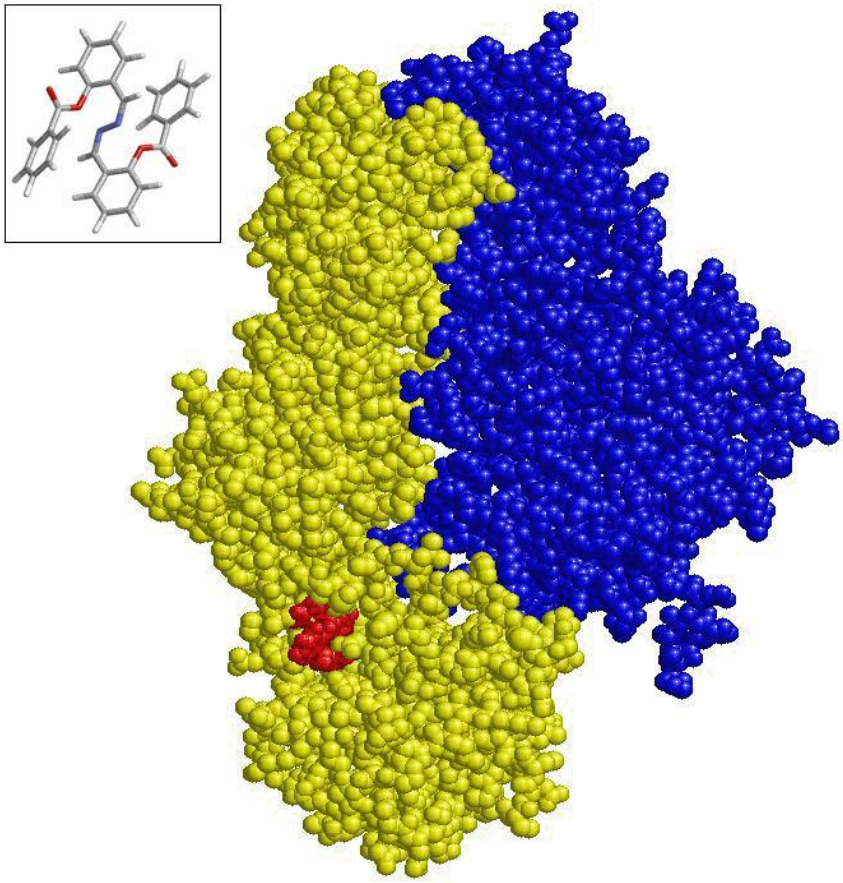

Figure 1. Structure of HIV1-RT complexed with HZ (Red). Subunits p66 and p51 are coloured yellow and blue respectively. Here both protein and ligand are shown in space-fill mode. Structure of HZ is shown in the inset. The coordinate of the HZ derivative was generated using the program Biopolymer of InsightII (MSI/Accelrys) (Honig et al., 1993; Nicholls and Honig, 1991; Sharp et al., 1991). Then energy minimization was done using Conjugate Gradient (CG) algorithm using the program DISCOVER (MSI/Accelrys) with consistent valance force field (cvff) until the derivative reached to 0.001kcal/mol (Dauber-Osguthorpe et al., 1988). The coordinates of enzyme RT were downloaded from the Protein Data Bank (ID: 2I5J). All the water molecules were removed and the protein coordinates were energy minimized using CG algorithm using the program DISCOVER (MSI/Accelrys) with cvff until the derivative reached 0.001kcal/mol. The structure which was energy minimized was used for superimposition and docking experiment. The energy minimised coordinates of hydrazine derivative was superimposed on the coordinates of the ligand, which was already present in 2I5J. The root mean square (r.m.s.) deviation was 0.085Å.The superimposed structure was again energy minimized using CG algorithm using the program DISCOVER (MSI/Accelrys) with cvff until the derivative reached 0.001kcal/mol in order to reduce the bad contacts.

termination and inhibition of viral replication. However, these nucleotide analogues can also get incorporated in the host DNA itself, causing damage to the host. Search for NNRTIs are therefore a favourable option. All known NNRTIs bind to the hydrophobic pocket of RT, but they do not prevent the binding of nucleic acid or nucleoside triphosphate substrates to RT; rather, the NNRTIs block the chemical step of the polymerization reaction (Rittinger et al., 1995; Spence et al., 1995).

Crystallographic studies have shown that the binding of the NNRTIs causes conformational changes by displacement of the 12, 13 and 14 sheet that contains the polymerase primer grip which is important for properly positioning the nucleic acid with respect to the polymerase active site (Rodgers et al., 1995). Binding of NNRTI in the hydrophobic pocket near RT polymerase active site can also influence its geometry and stops the polymerization reaction that eventually stops the HIV lifecycle (Ding et al., 1998). Examples of such drugs are like, nevirapine, efavirenz and delavirdine that bind near the

Table 1. To find the interactions between the HZ and the HIV-1 RT all the energy calculations were performed using Insight II software on a silicon graphics IndigoII workstation. HZ was also docked using AutoDock in order to get comprehensive result. The residues that are involved in ionic interaction with HZ are shown below. The amino acids showing H bonding and hydrophobic interactions are also indicated in the table.

Software used	Amino acids name with number
Insight II	VAL108, LEU109, ASP110, ASP185, ASP186, LEU187$^{¶+}$, TYR188, LYS223, PHE227^{+}, LEU228, TRP229, MET230, GLY231, LEU234, GLN 242
AutoDock 4	VAL108, ASP110, TYR181, GLN182, TYR183, ASP186, LEU187, TYR188, PRO217, PHE227, TRP229, LEU234

¶ denotes H-bonding. **+** denotes hydrophobic interaction.

primer grip region near the polymerase active site and inhibit polymerization (Balzarini, 2004; Smerdon et al., 1994). DHBNH, a hydrazone derivative, also binds to the hydrophobic pocket of RT, but it does not only acts as inhibitor of polymerization but also inhibits RNase H activity, although, its binding is away (<50 Å) from the RNase H activity site (Daniel et al., 2006).

MATERIALS AND METHODS

In the study's attempt to characterize the biological activity of a novel hydrazine derivative 2,2'- (diazinodimethylidyne) di-(o-phenylene) dibenzoate (Chattopadhyay et al., 2008), the authors have tried to investigate from theoretical studies if this molecule has therapeutic potential as HIV-RT polymerase inhibitor (NNRTI). For this purpose, they have generated the 3-D coordinates of the molecule and from docking experi-ments with HIV-1 RT. They have also shown that HZ binds in the hydrophobic pocket near the RT polymerase active site like the other NNRTIs. It interacts with all iden-tified amino acids that are important for catalytic activity of HIV1-RT, particularly those that are conserved. Our results indicate that HZ can act as a NNRTI by blocking the active site by interacting with the primer grip region and therefore has the potential act as anti- HIV drug.

RESULTS AND DISCUSSION

Both the two subunits of HIV1-RT (p66 and p51) consist of fingers (residues 1 - 84 and 120 - 150), palm (residues 85 - 119 and residues 151 - 243), thumb (residues 244 - 322) and connection (residues 323 - 437), but p66 has an extra domain, the RNase H domain (residues 438 - 556). There is an opening between the p66 fingers and thumb in which the non-nucleoside inhibitors can bind (Rodgers et al., 1995).

Comparison with the structures of HIV-RT and non-nucleoside inhibitor-complexed HIV-RT showed that only minor domain rearrangements occur, but there is a significant repositioning of a three-stranded beta-sheet in the p66 subunit which contains the catalytic aspartic acid residues 110, 185 and 186 (which resides in the palm of RT) (Esnouf et al., 1995). The 6- 9- 10 hairpin contains the polymerase active site and the residues that exist here are 105 - 112, 178 - 183 and 186 - 191 and the 12-13- 14 hairpin that contains the primer grip consists of the residues 227 - 229, 232 - 235 and 238 – 242 (Jacobo-Molina et al., 1993).

In all known structures of HIV-1 RT with NNRTI complexes, the position of the 12- 13 hairpin or the primer grip is significantly displaced relative to the position in the structure of HIV-1 RT complexed with a double-stranded DNA and in unliganded HIV-1 RT structures. Since the primer grip helps to position the template-primer, this displacement suggests that binding of NNRTIs would affect the relative positions of the primer terminus and the polymerase active site. This could explain biochemical data showing that NNRTI binding to HIV-1 RT reduces efficiency of the chemical step of DNA polymerization, but does not prevent binding of either dNTPs or DNA (Tantillo et al., 1994). The hydrazine derivative (HZ), (Figure 1) complexed with HIV-1 reverse transcriptase is shown in Figure 1. The residues that are present within 4 Å from HZ are shown in Table 1. These amino acids are a part of polymerase active site and primer grip region residues and are within the protein- ligand contact distance (Figure 2) (Diago et al., 2007). Therefore, the ligand has a strong ionic interaction with these residues.

In most cases, it is seen that the ligand-protein interaction site is highly mutative. Where the NNRTI binds the nearby amino acids gets mutated and some steric interactions come into play and disallow the ligand to bind at the active site of the RT polymerase active site (Smerdon et al., 1994). So, a drug designer must have a goal to invent such ligands that have a strong interaction with the conserved site residues, in this case, the ASP186 and TRP229. We found that HZ interacts with both of these two conserved amino acid residues (ASP186 and TRP229). HZ also additionally makes a hydrogen bond with LEU187. HZ also undergoes hydrophobic interactions with LEU187 and specifically with PHE227.

The hydrophobic side chain of LEU187 comes in contact with the C-skeleton of HZ. The aromatic rings of the HZ remains nearly stacked on to the aromatic ring of PHE227 thereby exposing the Diazino group to undergo H-bonding and other polar interactions with amino acids.

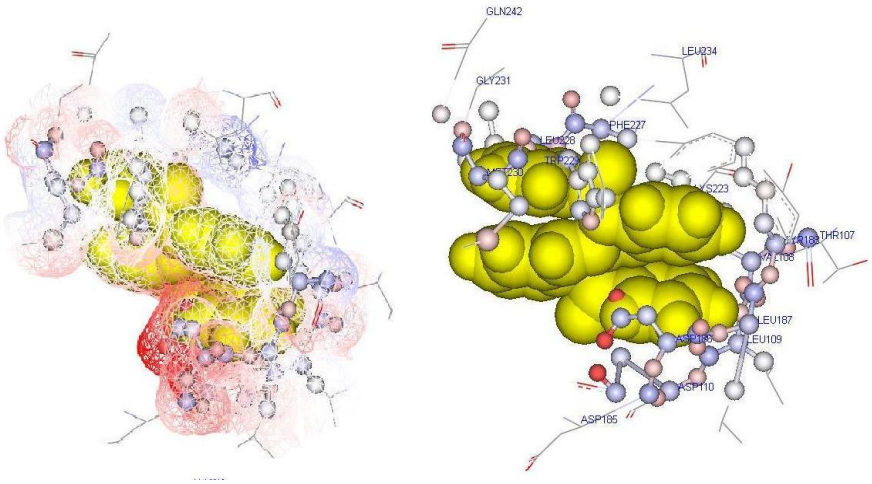

Figure 2. Pattern of charge distribution of residues within 4Å of HZ is shown in the left panel. The right panel shows the names of amino acid residues within 4Å from HZ. This calculation was done using Discovery Studio (2.0) (Honig et al., 1993; Nicholls and Honig, 1991; Sharp et al., 1991). These residues are shown in ball and stick in CPK colours and HZ is in yellow in space fill model. HZ also undergoes hydrophobic interactions with the protein. The hydrophobic interactions including stacking interactions were calculated using Insight II as well as WHAT IF server (Vriend, 1990) and Chimera (Pettersen et al., 2004).

A significant observation is that the conserved residues such as PHE227 and LEU234 (Smerdon et al., 1994) are also in close contact with HZ, implying that this new drug would be less vulnerable to escape mutations. Again from the free energy point of view that is obtained from the AutoDock (Morris et al., 1998) results, we have found that the best- docked free energy was -6.45 Kcal/mol (when the grid volume is 80), which indicates that ligand binds the RT polymerase active site very strongly.

Conclusion

This study has shown that HZ could work as a HIV1-RT inhibitor. The interaction of HZ with HIV1-RT is similar to the interactions of nevirapine, efavirenz, dihydroxy benzoyl naphthyl hydrazone and delavirdine, some well-known drugs in HIV treatment. However, as most of these drugs exhibit considerable side effects it merits search for new drugs. HZ does not only show interactions with all identified amino acids that are important for cata-lytic activity of HIV1-RT, but also with some other amino acids as well that are present in the catalytic domain. The free energy of binding also ensures that HZ shows a very strong binding with HIV1- RT at polymerase active site. Theoretical studies reveal that HZ has the potential for therapeutic use as HIV1 inhibitor.

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