Rational Drug Design Approach to Synthesizing HIV-1 Protease Inhibitors
By: Lingxian Ding
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Introduction

Human Immunodeficiency Virus (HIV), the causitive agent of AIDS, now infects an

estimated 20 million people worldwide, and while a cure has not yet been found for this fatal

disease, rapid advances in molecular biology along with the 3-D elucidation of HIV proteins

have led to new drug targeting approaches for designing antiviral agents that specifically bind to

key regulatory proteins that are essential for HIV replication. The HIV-1 protease is one such

enzyme crucial for the maturation and assembly of infectious viral particles. Thus by inhibiting

HIV-1 protease activity, a potential cure for AIDS is at hand. Armed with the 3D crystal

structure of the HIV-1 protease, rational drug design approaches have been widely employed in

the development of HIV protease inhibitors. While in clinical trials existent HIV-1 protease

inhibitors show promise in causing an initial dramatic decline in viral plasma RNA in HIV-

infected individuals, most patients taking protease inhibitors alone, however, by year's end will

show an increase in plasma viral RNA to near baseline levels (Kaplan 1996). Failure of HIV-1

protease inhibitor therapy is attributed to the development of resistant viral protease. While

techniques to combat resistance are currently being investigated, we have yet many obstacles to

clear before discovering a cure for HIV.

Structure and Function of HIV-1 Protease: Potential Drug Target Identified

Through X-ray crystallography techniques, the 3D structure of HIV-1 protease has been

extensively studied and characterized. In its mature form, the viral protease exists as a dimer,

whose subunits each consist of 99 amino acids. The folded subunits together interact to form a

core hydrophobic, cylindrical catalytic cavity (24Å long by 6-8 Å in diameter) and two flexible

flaps (one per subunit) that can close around the substrate. Centered in the hydrophobic active site are two symmetrically disposed catalytic aspartyl residues (Asp25 and Asp25') that are involved in hydrolysis of the peptide bond (West 1995). Studies have shown that the hydrophobic cavern can hold six amino acids of the substrate in an extended conformation for cleavage (Molla 1998). Because structural and enzymatic proteins of the HIV virus are translated as part of two polyprotein precursors (*Gag* and *Gag//Pol)* cleavage of these precursors to generate gag matrix (p17), capsid (p24), nucleocapsid (p15) proteins, *pol* reverse transcriptase, integrase enzymes, and other viral proteins is vital for the production of mature, infectious viral particles (Molla 1998). Since this cleavage process is critical to viral propagation, inhibition of viral protease proves to be an attractive drug target.

Rational Drug Design: Structure and Receptor Based Approaches

Initial approaches to developing HIV-1 protease inhibitors were based on characterizing substrate-protease interactions that led to cleavage. Studies by Griffiths, J.T. *et al.* (1992) found preference for proteolysis of substrates with scissile hydrophobic-hydrophobic (usually involving leucine, alanine, or valine) or aromatic-proline (i.e. phenylalanine-proline) peptide bonds. Hydrogen bonding and electrostatic interactions along the cylindrical groove were also taken into account. But beyond optimal fitting of amino acid side chains into the binding groove, characterization of transition state structures has also guided the design of protease inhibitors. In a functional protease a water molecule is held between the two active apartic acids. During hydrolysis the water molecule is added across the cleaved peptides. With this information at hand, the initial inhibitors consisted of small polypeptides of seven amino acids in length that were designed to mimic normal cleavage sites but with the replacement of a nonhydrolyzable isostere at the cleavage site. Furthermore, the central carbonyl groups of these potent protease

inhibitors also interact, via hydrogen bonding, with the water molecule held at the active site in order to stabilize the close formation of the flaps on the enzyme (West 1995).

While inhibitors such as Ro31-8959, LY289612, and other similar protease inhibitors designed with the above described criteria prove to be potent antivirals *in vitro,* they suffer from poor absorption, poor oral bioavailability, short serum half-life values, high susceptibility to hydrolysis by degradative enzymes, and other pharmacological problems (Reich 1995). Because of these limitations on peptidomimetic inhibitors, efforts have been made to minimize pharmacological problems by developing inhibitors that are structural mimics of peptides but with little or no peptidic character.

Reich *et al.* (1995) approached this problem by beginning with the cocrystal structure of HIV-1 protease with LY289612 (a peptidomimetic inhibitor). As their base design they retained only the non-peptidic hydroxyethyl-t-butylbenzamide portion of the original inhibitor. From here, the researchers synthesized and attached a variety of substituents to fit into the various active site pockets of the viral protease. By repeatedly solving the co-crystal structure of the protease and a newly designed non-peptide inhibitor (seven in all), they were able to improve upon their design and maximize binding affinities with each repetition. Further characterization *in vivo* showed improved bioavailability of up to 30% and maintenance in plasma levels, at or above, the antiviral IC_{90} for five or more hours in rats, dogs, and monkeys.

A different approach to designing non-peptide inhibitors involves the replacement of the water molecule at the catalytic site with a component of the inhibitor itself. In designing these compounds computer modeling techniques have been employed to generate a class of cyclic inhibitors (i.e. XM323). In XM323 and analogous compounds, the inhibitor's carbonyl oxygen is designed to displace the water molecule and hydrogen bond to Ile50. Cocrystalization of protease with the inhibitor confirmed computer generated predictions. While XM323 has 50% bioavailability and drug levels in plasma exceeds the IC_{90} for 4 to 16 hours, Phase I clinical trials

were abandoned in 1993 in favor of the derivative XM412 which was found to be more water soluble and bioavailable (West 1995).

As the above example demonstrates, in recent years computer simulation of molecular activity has become a powerful tool in rational drug design. Because of the availability of high-resolution of 3D structures of proteins, molecular dynamic computer simulations provide a means for studying rearrangement and interaction of these macromolecules at an atomic level. While in the past molecular dynamic simulations of HIV-1 protease were limited to 200 ps, recently long-term simulations have been achieved and confirmed by Ringhofer *et al*. (1999). In these experiments, emphasis was placed on predicting conformational changes induced by the inhibitor (SDZ283-910) as it bound to the active site. Judging the reliability of such simulations depended upon comparison of theoretical computer generated anisotropy decay data with experimental anisotropy decay data. Results confirmed close similarity between the two sets of data; thus affirming the potential for more extensive use of molecular dynamic simulation in the future to refine inhibitor optimization.

Combating Resistant Viral Variants

Despite rapid advances in designing potent protease inhibitors, their effectiveness has been hampered by the emergence of drug-resistant viral variants. Because of rapid viral replication and high error prone rates of HIV reverse transcriptase (about 1 in every 10,000 bases), mutations that confer drug resistance are highly favorable (Erickson 1996). Mutations observed to date have been clustered around the active site in which the introduced mutation reduces inhibitor binding potential by either reducing contact between the inhibitor and the protease (reducing van der Waal interactions) or by competing for inhibitor space (West 1995). But interestingly enough non-active site mutants have also been characterized at the hinge region. The mechanism for drug resistance of these mutants are not yet clear but a reasonable

hypothesis is that they indirectly induce conformational changes at the active site. Finally, studies have also characterized cleavage site mutants. Since mutations at the active site can affect the rate at which the *Gag* and *Gag/Pol* protein precursors are cleaved by the protease, one can imagine that mutations at the cleavage site of these respective protein precursors could compensate for reduced mutant activity. In effect, mutations at the cleavage site of the precursor protein could render them as better substrates for the mutant protease. Recent studies have identified the existence of such mutants. Studies show that a mutation in the p1-p6 *gag* polyprotein cleavage site, in which the wildtype Phe-Leu cleavage site is replaced by a Phe-Phe, renders the 184V mutant protease capable of producing a virus with 10^2 to 10^3 fold decreased sensitivity to an inhibitor, Bila 2011 (Erickson *et al*. 1996).

But because most drug resistant mutations occur at the active site, in theory, only a limited number of mutations can be supported by the virus since random mutations can render the protease non-functional. Thus by understanding the structural basis for resistance, new drugs can be designed to target major classes of mutants. For example, a "structured-directed oscillation therapy" can be developed to combat resistance. In this approach, patients will be treated with a combination of protease inhibitors designed to simultaneously target both the mutant and wild-type enzyme. The drugs will be applied in such a fashion that selection pressure mutates the protease back and forth between wild-type enzyme and treatable mutant variant (Erickson 1996). Also studies by Molle *et al*. (1996) and Kempf *et al*. (1998) showed that resistance arises because viral replication is incompletely suppressed in the plasma or tissue during periods of time between doses when drug concentrations are lowest. Thus development of more bioavailable inhibitors that sustain high plasma levels are also needed to combat resistance.

Conclusion and Future Direction

While rational drug design techniques have led to the development of many classes of potent HIV-1 protease inhibitors, problems such as viral resistance, moderate bioavailability, short plasma half-life continue to obstruct the path toward finding a cure. Computer simulations predict that a mutant virus with a 5% selective advantage (i.e. a replication rate 1.05 times that of the wild-type virus) will become the dominant genotype within 100 virus generations (Erickson, 1996). While this data may seem daunting at first, one must keep in mind that with more and more powerful computer simulation programs, it will perhaps become possible not only to visualize mutant viral particles but also to predict the array of possible mutations that a particular drug might induce. This capability will aid in designing an effective "structure-based oscillation therapy." Besides combating resistance, current HIV-1 protease inhibitors in development also need to address pharmacokinetics issues. Continued advances in rational drug design methodologies hold promise for solving the above mentioned obstacles.

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