Novel Binding Site Targeting HIV-1 RNase H May Lead to Future Anti-HIV Drug Design

By Ashley DeVine, Staff Writer

Although a variety of drugs exists to treat HIV, a major concern is drug resistance, which requires researchers to constantly identify new drugs with enhanced potency and specificity.

Research has demonstrated that the ribonuclease H (RNase H) activity associated with HIV-1 reverse transcriptase (RT) is essential for virus replication, yet no drugs inhibiting this function currently exist.

A study led by Suhman Chung, Ph.D., a postdoctoral CRTA in the Reverse Transcriptase Biochemistry Section, HIV Drug Resistance Program, investigated two relatively potent RNase H inhibitors, called vinylogous ureas, to better understand where they bind to HIV-1 RT to inhibit RNase H.

Previous research proposed that the vinylogous urea, NSC727447, allosterically inhibited the RNase H activity of HIV-1 RT by interacting with the thumb subdomain of its non-catalytic p51 subunit. HIV-1 RT is a heterodimer composed of two subunits, p66 and p51. The RNase H active domain is located in p66, and the thumb subdomain of p51 is in close proximity to p66. Chung and colleagues believe that the binding of these inhibitors may affect the architecture of the RNase H active site.

“Developing allosteric inhibitors of RNase H activity is particularly important since finding drugs like active-site inhibitors has been challenging,” Chung said. “This study is so far the only source of valuable information on residues involved in inhibitor binding and provides useful data for future drug design as well as important mechanistic insight of the inhibition.”

Chung, who began working at NCI-Frederick in September 2007, earned his Ph.D. in bioorganic chemistry from POSTECH in South Korea.

Mutagenesis of the Human Immunodeficiency Virus Reverse Transcriptase p51 Subunit Defines Residues Contributing to Vinylogous Urea Inhibition of Ribonuclease H Activity

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The vinylogous urea, NSC727447, was proposed to allosterically inhibit ribonuclease H (RNase H) activity of human immunodeficiency virus Type 1 reverse transcriptase (HIV-1 RT) by interacting with the thumb subdomain of its non-catalytic p51 subunit. Proximity of the p51 thumb to the p66 RNase H domain implied that inhibitor binding altered active site geometry, while protein footprinting suggested a contribution from alpha-helix I residues Cys280 and Lys281. To more thoroughly characterize the vinylogous urea binding site, horizontal alanine scanning mutagenesis between p51 residues Lys275 and Thr286, (comprising alpha-helix I and portions of the neighboring alphaH/alphaH and alphaH/alphaJ connecting loops) was combined with a limited vertical scan of Cys280. A contribution from Cys280 was strengthened by our observation that all substitutions at this position rendered selectively mutated, reconstituted p66/p51 heterodimers ~45-fold less sensitive to inhibition. An ~19-fold reduced IC50 for p51 mutant Thr286Ala, coupled with a 2- to 8-fold increased IC50 when intervening residues were substituted, supports our original proposal of p51 alpha-helix I as the vinylogous urea binding site. In contrast to these allosteric inhibitors, mutant enzymes retained equivalent sensitivity to the natural product alpha-hydroxytropolone inhibitor manicol, which x-ray crystallography has demonstrated functions by chelating divalent metal at the p66 RNase H active site. Finally, reduced DNA strand-transfer activity, together with increased vinylogous urea sensitivity of p66/p51 heterodimers containing short p51 C-terminal deletions, suggests an additional role for the p51 C-terminus in nucleic acid binding that is compromised by inhibitor binding.