147

Boron Modified Peptide: a Novel Dissociative Inhibitor of HIV-1 Protease.

Arman D. Pivazyan & William H. Prusoff, Department of Pharmacology, Yale University, New Haven, CT 06520-8066

HIV-1 protease is a key enzyme in HIV maturation and is responsible for posttranscriptional processing of viral fusion polyproteins. This process is necessary for the release of active reverse transcriptase, protease itself and other essential viral proteins. Inhibition of the HIV-1 protease leads to the production of noninfectious viral particles.

We examined the inhibition kinetics of this protease by a boron modified tetrapeptide, Ac-Thr-Leu-Asn-Phe-Boronic acid, which is the terminal analog of the HIV-1 gag-pol polyprotein scissile bond (Phe-Pro), and previously found to be an inhibitor of HIV-1 protease. Two heptapeptides were used as substrates, and the enzyme kinetics was followed by HPLC. This boron modified analog did not show traditional Michaelis-Menten inhibition kinetic patterns. Analysis of the data showed that the inhibition was related to the composition of the reaction mixture (enzyme, substrate and inhibitor) and could be either dissociative or competitive. In the presence of the inhibitor the Kd of the enzyme is about $2x10^{-7}$M and in the absence of the inhibitor is about 3-times lower. The Ki for the competitive type of inhibition is about $5x10^{-6}$M. Because of the dual type of inhibition the apparent Ki for the boron peptide varies from 10^{-7}M to $5x10^{-6}$M and depends upon the composition of the reaction mixture..
Non-boronylated peptides have been reported to be dissociative inhibitors of HIV-1 protease, but at concentrations at least 30 to 1000 times higher (Schramm et al. (1993), BBRC V194, #2, 595-600). The boron-tetrapeptide analog is considered to be a lead compound. Thus boron modified peptides can be strong dissociative inhibitors of HIV-1 protease with dual type of inhibitory action, and hence represent a novel approach for development of drugs for therapy of AIDS.

Supported by USPHS, NIH grant CA-05262 from the NCI.

148

Factors Other Than Reverse Transcriptase Contribute to Resistance of Feline Immunodeficiency Virus to Reverse Transcriptase-Targeted Antivirals. T. W. North, K. M. Remington, Y.-Q. Zhu, R. A. Smith and D. G. McBroom, Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA.

Two lines of evidence support the involvement of viral components other than reverse transcriptase (RT) in resistance of feline immunodeficiency virus (FIV) to RT-targeted antivirals. The first is from studies of a 3'-azido-3'-deoxythymidine (AZT)-resistant mutant of FIV, AZR-17c, which has a single base change resulting in substitution of lys for glu at position 202. RT purified from this mutant was resistant to the 5'-triphosphate of AZT. We have isolated a phenotypic revertant of AZR-17c which, despite wild-type sensitivity to AZT, retains the mutation resulting in lys 202. Moreover, RT purified from this phenotypic revertant remained resistant to the 5'-triphosphate of AZT. There are no other mutations in the RT-encoding region of AZR-17c or the revertant. Sequence analysis to identify the mutation responsible for suppression of the AZT-resistant phenotype will be presented. The second line of evidence for involvement of factors other than RT in drug-resistance is from studies of an FIV mutant, PFR-2, that is resistant to phosphonoacetic acid (PFA). The RT detected in preparations of detergent lysed virions derived from PFR-2 was resistant to PFA, whereas the purified RT from this mutant was not resistant to PFA. We hypothesize that resistance to PFA requires interaction of the FIV RT with another protein. (Supported by NIAID grant AI-28189)