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Initial Binding of 2'-Deoxynucleoside-5'-Triphosphates to HIV-1 Reverse Transcriptase. G. Painter, L. Wright, S. Hopkins, P. Furman, Wellcome Research Laboratories, Research Triangle Park, NC, USA

HIV-1 reverse transcriptase, a heterodimer consisting of two polypeptide chains of molecular weights 66 kD and 51 kD has an intense fluorescence emission peak centered at 338 nm due to the presence of 36 tryptophan residues. The association of 2'-deoxynucleoside-5'-triphosphates (dNTPs) with the enzyme results in a decrease in the intensity of the tryptophan emission spectrum that can be used to calculate apparent K_d values. The K_d values determined for binding of the four natural dNTPs to the enzyme range from 3.67 X 10^{-5}M for dTTP to 4.73 X 10^{-5}M for dATP. The 5'-triphosphate of the zidovudine has a K_d of 5.41 X 10^{-5} M. The enzyme shows no preference for either purine or pyrimidine nucleotides. Hill coefficients derived from fluorescence titration data and the results of dual ligand titration experiments demonstrate that the enzyme possess a single dNTP binding site for which the four natural dNTPs and the 5'-triphosphate of zidovudine compete. The presence of homopolymeric template-primers does not result in selective binding of the complementary dNTPs indicating that Watson-Crick base pairing is not involved in the initial binding reaction. The major force driving the as-sociation of the ligands with the binding site is hydrophobic as evidenced by the minimal effect of ionic strength on the apparent K_d of dTTP. Approximately 15% of the binding energy is derived from electrostatic interactions. Although Mg^{+2} is required for catalytic activity, it is not required for initial binding.

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SYNTHETIC INHIBITORS OF HIV-1 PROTEASE INHIBIT VIRUS MATURATION AND INFECTIVITY OF VIRIONS. D.M. Lambert, J. Leary, T.J. Matthews*, T.D. Meek, G.B. Dreyer, T. Hart, B.W. Metcalf and S.R. Petteway, Jr. Departments of Anti-Infectives, Medicinal Chemistry, and Experimental Pathology, SmithKline & French Laboratories, 709 Swedeland Road, King of Prussia, PA 19406-0939; Department of Surgery, Duke University Medical School, Durham, NC 27710.

Introduction. The effectiveness of HIV-1 protease inhibitors as antiviral agents requires that the resultant inhibition of polyprotein processing within infected cells be translated to a profound reduction in viral infectivity. Synthetic peptide analog inhibitors of HIV-1 protease were used to study the effects of inhibiting polyprotein processing on the assembly, structure and infectivity of virions released from chronically infected T-lymphocyte cell cultures.

Methods/Results. Using Western blot and immune precipitation techniques, we have shown that virions from treated cell cultures contained $Pr55^{gag}$ and processing intermediates. A corresponding diminution of the mature protein products p24 and p17 was seen. Virions also contained reduced reverse transciptase activity and increased amounts of gag-pol precursor polyproteins. The infectivity of virion particles produced by these cells was followed over a 3 day period of inhibitor treatment. By day 2 of treatment, >100-fold reduction in $TCID_{50}$ titers was observed and by day 3, no infectious virus could be recovered. Moreover, electron microscopy revealed that virtually all of the virion particles produced from inhibitor-treated cells were of an immature morphology. This morphologic change correlated with the inhibition of polyprotein processing and loss of infectivity. These results clearly demonstrate that HIV-1 protease inhibitors exert a potent antiviral effect on chronically infected T-lymphocytes by blocking the production of infectious virions.