39

ACCURATE QUANTITATION OF HIV DNA AND RNA SEQUENCES USING QUANTITATIVE COMPETITIVE POLYMERASE CHAIN REACTION (QC-PCR)

*M. Piatak, *K.-C. Luk, **M. McGrath, ***B. Williams and *J. Lifson; *Genelabs Inc., Redwood City, CA, USA; **University of California, San Francisco, and San Francisco General Hospital, San Francisco, CA, USA; ***Applied Imaging, Inc., Sanat Clara, CA, USA

Variations in the absolute yield of amplified PCR product from replicate or sequence equivalent samples may result from differential kinetics due to the amount of input target, the prescence of inhibitors, or amplification of background products. Co-amplification of a heterologous target sequence as an internal control does not optimally address these concerns, given potential differences in relative template amounts and priming kinetics. A recently developed competitive PCR method (Gilliland et al, PNAS, 87:2725), based on its inherently competitive nature, obviates these problems. We have adapted this approach to achieve accurate quantitation of HIV DNA and RNA sequences. The quantitative competitive-PCR (QC-PCR) technique is based on the principle of determining the equivalence point for the amounts of PCR product derived from an unknown amount of wild type target sequence and from known amounts of a nearly identical, but electrophoretically discriminable mutant competitive template added to the same reactions. The same primers amplify sequences from both wild type target and the added competitive template. Primers incorporate inosine residues to account for potential sequence divergence among viral isolates in the highly conserved target region. For RNA PCR, an RNA competitive template can be added to aliquots of the test sample to provide an internal control for the reverse transcription reaction that precedes the PCR. In vitro studies using serial dilutions of the clonal 8E5/LAV cell line (single integrated proviral copy per cell) or serial infectious virus stocks indicate that QC-PCR can detect and accurately quantitate as few as 10 copies of HIV DNA or RNA. We have successfully applied this technique to the quantitative HIV DNA and RNA in blood specimens from infected patients. This procedure may be used to monitor HIV replication in vitro in cell types available in limiting numbers. Accurate quantitative monitoring of viral load in vivo in response to treatment may be an especially promising application of the technique.

40

Measurement of Association and Dissociation Rate Constants of HIV Protease Inhibitors Using Stopped-Flow Fluorescence. E. S. Furfine+, E. D'Souza@, K. Ingold#, J. J. LeBan#, D. J. T. Porter+, T. Spector+, and J. E. Reardon+. Divisions of Experimental Therapy+ and Organic Chemistry#, Wellcome Research Laboratories, Research Triangle Park, North Carolina USA, Division of Molecular Sciences@, Wellcome Research Laboratories, Beckenham, UK

Since human immunodeficiency virus (HIV) protease is required for maturation of infectious HIV particles, it is a potential target for chemotherapeutic treatment of acquired immunodeficiency syndrome (AIDS). Numerous potent inhibitors of the protease have been synthesized. To understand the mechanism of these inhibitors, we examined the binding of a transition state mimetic inhibitor quinoline-2-carbonyl-Asn-PheΨ[CH(OH)CH_2N]Pro-O^{t}Butyl (1), which quenches the fluorescence of the HIV protease. The fluorescence perturbation was utilized to determine the association and dissociation rate constants by stopped-flow fluorometry. Further, the dissociation rate constants for three additional inhibitors (benzoyloxycarbonyl-PheΨ[CH(OH)CH_2N]Pro-O^{t}Butyl (2), Acetyl-Thr-Ile-Leu[CH_2NH]Leu-GLn-Arg-NH_2 (3), and pepstatin) were determined by trapping enzyme as it was released from the enzyme·inhibitor complex with 1. Association rate constants of 2, 3, and pepstatin were determined by pre-steady-state kinetics of the protease with the fluorescent substrate 2-Aminobenzoyl-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg-NH_2 (4). Dissociation constants that were calculated from the association and dissociation rate constants were similar to the K_i values determined by steady-state kinetic analysis with substrate 4.