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Expression and Characterization of the Retroviral Protease of the Simian Immunodeficiency Virus. Ingrid Deckman, Stephen Grant, Jeffrey Culp, Michael Minnich, Thomas Meek and Christine Debouck, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, USA.

The search for an animal model for AIDS has prompted the detailed investigation of other animal retroviruses. Simian Immunodeficiency Virus (SIV), a close relative of HIV-1 displays regions of well-conserved homology throughout its gag, pol and env proteins. The retroviral proteases of SIV and HIV-1 exhibit 85% conserved homology, but identity of only 45% limited to the active sites, flaps and alpha-helical regions of the proteins. In an effort to explore the utility of SIV as a model to evaluate HIV-1 protease inhibitors, the aspartyl protease of SIV was expressed in E. coli. We verified and characterized the SIV protease activity using a double plasmid system in E. coli for the co-expression of the SIV protease and various polyprotein substrates. The SIV protease was purified and further characterized by measuring Km's of small peptides corresponding to natural cleavage sites of HIV-1 protease. Due to insolubility of some of the peptides, a novel approach was developed to measure relative Km's of these cleavage sites by genetically engineering these sites into an assayable enzyme system. Finally, a variety of protease inhibitors, including pepstatin and SKF HIV-1 protease inhibitors, were tested on the SIV protease to determine the applicability of this retrovirus to the evaluation of HIV-1 protease inhibitors.

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Molecular Targets for the Development of AIDS Therapeutics. C. Debouck, Department of Molecular Genetics, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, USA.

One of the modern strategies for the development of treatments for infectious diseases involves the recombinant expression and functional analysis of one or more genes that are essential for the infectious agent life cycle. Once specific assays are established for these essential functions, they are used to identify anti-infective compounds by random screening and/or rational design. We have applied this type of strategy to the search for anti-viral compounds active against the human immunodeficiency virus (HIV). The molecular target that we focused on is the retroviral protease (PR), but we have also studied to some extent the HIV-1 reverse transcriptase (RT) and tat transactivator. We have expressed active PR, RT and tat in the bacterium Escherichia coli and purified these proteins to homogeneity. Specific enzymatic assays were established for PR and RT, and a specific cellular adhesion assay was developed for tat. We showed that PR can proteolytically cleave at least 8 sites within the HIV-1 gag-pol, including the site corresponding to the C-terminus of the p51 chain of the RT heterodimer. Peptide mimics of the HIV-1 p17-p24gag cleavage site were synthesized and shown to exhibit antiviral activity in in vitro infected cells. The current status of research on these molecular targets will be presented.