A-11: Human immunodeficiency virus. proteinase specificity and screening procedure to detect its inhibition

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Mutations in the proteinase coding region of the human immunodeficiency virus (HIV) genome have been shown to result in production of non-infective virons. Thus HIV proteinase has been targeted as a possible therapeutic intervention point in the treatment of AIDS. Therefore it is important to investigate the cleavage specificity of HIV proteinase to develop an assay procedure for the screening of inhibitors of proteinase activity.

The chemically synthesized gene of HIV proteinase was cloned into BamHI site of the T7 expression vector pET3b and expressed in *E. coli* cells. The

expressed product was purified from inclusion bodies and refolded to proper conformation with proteolytic activity.

The cleavage specificity of HIV proteinase was investigated at pH 5.5, 6.5 & 7.4, using the B chain of oxidized insulin as a substrate. Highest activity was found at pH 5.5. The cleavage sites and extent of hydrolysis were estimated by amino acid analysis. Digestion at pH 5.5 cleaved two peptide bonds Glu (13)-Ala(14) and Tyr(16)-Leu(17) to marked extent, whereas at pH 6.5, the Tyr(16)-Leu(17) was cleaved fairly selectively. The extent of hydrolysis at pH 6.5 was 33.3% and at pH 7.4 was 0.0% when compared to the activity at pH 5.5.

The results suggest that B chain of oxidized insulin is a good substrate for HIV proteinase and digestion at pH 5.5 followed by HPLC is a good procedure to screen for inhibitors of HIV proteinase activity.