

Design and characterization of macromolecular inhibitors of human immunodeficiency virus 1 protease

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We have developed a high throughput microtiter plate fluorescent assay which can be a good alternative to the traditional HPLC-based protease assay, since it is faster and is able to measure several parallel samples. Our assay was validated by using the traditional method and applied to HIV-1 and HTLV-1 proteases. Several new fluorescent substrates containing EDANS/DABCYL groups were designed and tested. We have also developed an alternative method for the inner filter correction. Inhibition profiles of HIV-1 and HTLV-1 PRs were compared using various clinically used HIV-1 PR inhibitors and new HTLV-1 PR inhibitors were also designed and tested. Later, the new method has also been applied successfully for additional retroviral proteases.

Furthermore, we have designed and investigated defective HIV-1 proteases which contained mutations in the active site and in the substrate binding site. After the *in silico* mutagenesis, we prepared and purified selected proteins for *in vitro* as well as in cell culture experiments to determine their inhibitory effect on wild-type HIV-1 PR. Mutants containing charged residues showed dose-dependent, specific, trans-dominant inhibitory effect in our experiments, and they represent a new generation of macromolecular inhibitors against HIV-1 PR. Heterodimerization could be detected, for the first time, between a wild-type retroviral PR and trans-dominant negative mutants by NMR spectroscopy using our novel hydrophilic constructs and our facile protein folding protocol. The Asp25Arg and the Gly49Glu mutations introduced into analogous positions of other retroviral PRs may exert a similar inhibitory effect on the corresponding wild-type PR. These residues target the catalytic aspartate, thus it may be considered a general strategy for all retroviral PRs.

Key words: HIV-1 protease, fluorescent assay, macromolecular inhibitor

Kulcsszavak: HIV-1 proteáz, fluoreszcens esszé, makromolekuláris inhibitor