

Characterization of the Bovin Leukemia Virus Protease and Comparison with Other Retroviral Proteases

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During my Ph.D work I had the opportunity to study the BLV proteinase in details and to compare its features with other retroviral proteases (mainly with HIV-1 and HTLV-1 proteases).

We built a molecular model of the BLV PR. The specificity of the BLV PR was characterized using a large set of oligopeptides representing naturally occurring cleavage sites in various retroviruses. Based on the results, the BLV PR appears to have a broad substrate specificity, similar to HIV-1 PR, but unlike the related HTLV-1 PR. The broader specificity of the BLV PR, compared to the HTLV-1 PR, was also verified by mapping the individual substrate binding sites using a set of oligopeptides with single amino acid substitutions. Furthermore, the substrate binding site of BLV PR appeared to be less extended compared to that of HTLV-1 PR. While both BLV and HTLV proteases showed a preference for larger hydrophobic P2 and P1 residues, BLV PR much better tolerated hydrophilic or even charged residues at these positions. Nevertheless, in most aspects the specificity of individual subsites of BLV PR more closely resembled that of HTLV-1 PR, in good agreement with the more similar sets of residues predicted to be involved in substrate binding, as compared to those of HIV-1 PR. It appears to be a common characteristic of BLV and HTLV-1 proteases that their folding capability and/or catalytic efficiency are much more sensitive towards mutations than many other retroviral proteases, especially HIV proteases. Inhibition profile of the BLV PR resembles more to that of HTLV-1 PR as compared to HIV-1 PR. However, BLV PR was substantially better inhibited as compared to the HTLV-1 PR. This effect appears to correlate with the generally lower K_m values observed for BLV PR as compared to HTLV-1 PR.

The specificity of the critical S2 substrate binding site of the proteases of eleven retroviruses representing each of the seven genera of *retroviridae* was studied using a series of oligopeptides having amino acid substitutions in P2 position. Despite the previous experiment, when we employed oligopeptides based on naturally occurring type 2 cleavage site sequences, the S2 binding site of BLV PR appeared to be a relatively large pocket and a preference for Leu was observed, without considerable hydrophilic residue tolerance. However, it is important to note that the specificity of retroviral proteases appears to be strongly context dependent, as reviewed for HIV-1 PR.

The specificity distinction of the proteases correlated well with the phylogenetic tree of retroviruses prepared solely based on the PR sequences. Molecular models for all studied proteases were built, and they were used to interpret the results. While size complementarities appear to be the main specificity-determining features of the S2 subsite of retroviral proteases, electrostatic contributions may play a role only in case of HIV proteases. In most cases the P2 residues of naturally occurring type 1 cleavage site sequences of the studied proteases agreed well with the observed P2 preferences.

In conclusion, based on our studies, despite the specificity differences, in terms of mutation intolerance and inhibitor susceptibility of the PR, BLV and the corresponding animal model systems may provide good models for the test of PR inhibitors that would be developed by in vitro studies against the protease of HTLV-1. Understanding the specificity similarities and differences of the retroviral enzymes may help to design broad-spectrum inhibitors against HIV-1 PR.