Abstract

Amino acid changes within HIV protease or its substrate that decrease the susceptibility to protease inhibitors represent a highly complex issue still not yet fully understood. Various mechanisms by which this often complicated pattern of mutations influence drug binding needs to be analyzed on a molecular level by a series of methods including experiments with recombinant viruses, biochemical enzyme analysis, structural and thermodynamical studies or molecular dynamics. Each result may help to complete the overall picture of protease inhibitor resistance evolution and therefore contribute to the design of more powerful 3rd generation HIV/AIDS drugs.

This thesis presents several analyses of HIV resistance development on molecular level. We have focused on the nelfinavir resistance pathway, lopinavir mutation score, emergence of amino acid insertions in HIV protease gene and their contribution to protease inhibitor resistance and finally we analyzed a highly mutated protease species isolated from patients failing darunavir therapy. Since we are able to accomplish a wide combination of techniques, we could explain and put together some pieces of viral evolution considering the final steps of HIV life cycle and also provide knowledge necessary for novel inhibitor design.

Aims of the Project

There were four major aims of the project that all had one goal in common: to investigate different mechanisms leading to resistance development to HIV protease inhibitors. The first aim was to analyze evolutionary pathways leading to resistance towards protease inhibitor nelfinavir and to inspect the role of compensatory mutations in terms of *in vitro* kinetics, structural and thermodynamical analyses and computational dynamics. Secondly, we aimed to inspect lopinavir mutation score, specifically the mutation I47A in and out of the background of other mutations isolated from a patient; and to find synergistic accompanying substitutions and analyze them on the molecular level. The third aim was to explore a possible novel mechanism of resistance development by incorporating amino acid insertions into the protease. Our goal was to analyze patient-derived proteases with one amino acid insertion at position 33 and 35 in terms of *in vitro* kinetics and crystal structures and to analyze PR susceptibility and replicative capacity of corresponding viruses *in vitro*. The last aim involved proteases carrying multiple drug-resistant mutations isolated from the patients failing darunavir therapy. Our intention was to investigate the parameters driving to resistance towards darunavir in terms of enzyme kinetics, structural analyses and thermodynamical characteristics.

Conclusions

In the first published paper of this thesis we focused on two different pathways driving nelfinavir resistance along D30N and L90M substitutions. We also investigated various effects of compensatory mutations, all by means of *in vitro* kinetic measurement, thermodynamical analysis, X-ray structures and molecular dynamics. We learned, apart from many specific results, that only the combination of several molecular techniques can help to fully analyze the problem. Second paper guided us into lopinavir resistance evolution. Lopinavir, as a second generation inhibitor designed to inhibit resistant viral species, is widely used with very good results. Understanding its complex resistance profile was a major challenge for us. We described structural changes resulting from introducing I47A mutation into the backround of patient-derived substitutions and explained them also with the help of energy calculations. The project summarized in the third paper was a truly "cherry on top" for us. We looked closely into the problem of amino acid insertions, investigated them in terms of enzyme kinetics, viral replication and structural analysis and we happened to be the first who solved the structure of HIV-1 protease with an insertion. We also showed that insertions in the protease gene might represent completely novel mechanism of resistance development to protease inhibitors. Finally, the data published in the fourth paper again proved that the better the inhibitor the more complicated its resistance profile is. Darunavir is a protease inhibitor with high genetic barrier to resistance development and requires accumulation of considerable number of mutations for the resistance to occur. We closely looked into darunavir mutation score, a set of PR mutations responsible for the viral resistance to darunavir, by analyzing patient-derived proteases harboring record-breaking number of mutations. We were not only able to produce them is sufficient amount and purity for crystallization experiments, but also we succeeded in crystallizing two of those in complex with darunavir and show the structural differences causing the resistance.

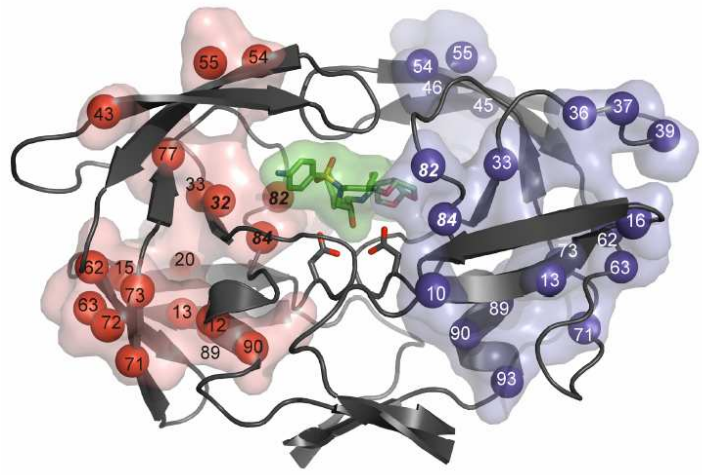

Figure 1: Positions of the mutations in protease variants used for structural studies concerning darunavir resistant variants.