Abstract

HIV-1 protease plays a crucial role in the late state of the life cycle of HIV virus when it cleaves the viral polyprotein precursors into the structural and functional proteins. If it is effectively inhibited, HIV particles remain immature and noninfectious. The application of highly active antiretroviral therapy (HAART) including protease inhibitors can reduce plasma HIV-1 levels below the detection limit in adherent patients and thus dramatically change their life expectancy. The clinical utility of the first inhibitors was limited by severe side effects, low bioavailability, high pill burdens, and rapid development of viral resistance under the selection pressure of HIV antiretrovirals. To overcome these difficulties, second-generation inhibitors were developed. Despite an indisputable improvement they brought to antiretroviral therapy, the development of new highly active HIV-1 protease inhibitors with optimal pharmacokinetic properties, higher metabolic stability, little off-target activity, and particularly, more favorable resistance profiles is still of high importance.

This thesis provides an overview of anti-HIV- drugs including development of substituted metallacarboranes, a new class of potent, unusual, nonpeptidic HIV protease inhibitors with therapeutic potential.

Next, the impact of protease background on the development of resistance of maturation inhibitor bevirimat is analyzed. Our data suggest that the mutations in the protease influence the level of antiviral resistance towards bevirimat. The viruses with mutated proteases show more diverse resistance profiles compared to those with wild-type protease. These observations can be explained by the different efficiencies of the Gag substrate cleavage by the different proteases.

Finally, regulation of enzymatic activity by small alkali cations has an important role in many biological processes. Their specific effects on the HIV protease activity were studied by a combination of experimental and computational techniques. Our molecular dynamic simulations confirm that the affinity of alkali cations to the HIV protease surface follows the Hofmeister series, mostly due to interactions with carboxylate side chain groups of aspartates and glutamates. Accordingly, our experimental data also showed that the initial velocity of peptide substrate hydrolysis in the presence of different alkali cations generally follows the Hofmeister series, with the exception of caesium. The higher catalytic efficiencies (k_{cat}/K_M) in the presence of K^+ ions in comparison to other alkali cations were observed at corresponding salt concentrations. Furthermore, we observed an unexpected increase in the hydrolysis of a specific substrate at very low salt concentration.