ABSTRACT

The inhibition of HIV-1 protease plays an important role in combating HIV. Nine HIV-1 protease inhibitors have been succesfully marketed for the treatment since 1995. However, their efficiencies decrease due to the resistance development. More potent compounds with novel structural motifs and mechanisms of action are therefore still needed. Several inhibitory compounds have been reported to bind to the protease at the loci different from the active site.

Interestingly, darunavir, which is the last approved inhibitor with supposedly competitive mode of action, was also suggested to bind to the flap region of the protease. Two studies discussed this alternative binding mode based on the X-ray structural and kinetic analysis, respectively. Nevertheless, it is questionable, if such a mechanism is relevant also in physiological conditions or if it is only an artifact of crystallization. Another study provided a strong evidence for the alternative binding of darunavir to highly mutated HIV-1 protease. Based on thermodynamic analysis, it was shown that two molecules of darunavir bind to the protease dimer. Surprisingly, this observation was not confirmed by the X-ray structure analysis since the inhibitor was bound only within the active site. However, this protease variant was employed in further studies.

To study the mechanism of darunavir binding to HIV-1 protease, several alternative approaches we choose including nuclear magnetic resonance (NMR) and surface plasmon resonance (SPR) methods as well as kinetic analysis using spectrophotometric assay. For these purposes, the panel of eleven isotopically labeled or tag-extended HIV-1 PR variants was prepared and characterized within this diploma thesis. The proteins were expressed, purified and characterized. The influence of the mutations and the tag-extension on the stability and activity of HIV-1 protease was investigated. Based on the kinetic analysis using spectrophotometric assay, the mechanism of inhibition of mutated protease by darunavir was suggested as that of the mixed type.

This study represents the background for further investigation of the alternative binding of protease inhibitors.

Key words: binding site; enzyme kinetics; HIV protease; protein structure; affinity tag