

Gag polyprotein is the precursor of HIV-1 structural proteins, required for correct assembly, budding and maturation of viral particle within HIV-1 life cycle. The process of maturation into an infectious virion is dependent on Gag and GagPol cleavage at nine predefined sites by HIV-1 proteinase. Its disruption is one of the main targets of HIV treatment. HIV-1, however, develops resistance to the proteinase inhibitors by creating mutations in both the proteinase and the substrate.

The Gag processing by HIV-1 proteinase is a highly sequential process, that happens in specific order and rate. Previous biochemical studies determined the kinetic data of these processes using oligopeptides representing naturally occurring cleavage sites. This thesis describes the cleavage of the Gag polyprotein itself, which is the natural substrate of HIV-1 proteinase. For this purpose, the full-length Gag polyprotein was recombinantly prepared in bacterial expression system. The cleavage was carried out and its products were analyzed via SDS-PAGE and Western blotting. The substrate specificity of the wild-type and mutant HIV-1 proteinase with respect to the full-length wild-type Gag polyprotein was compared. Substantial differences were observed between the rates of individual steps of cleavage by the wild-type and mutant HIV-1 proteinase.